FEDERATION PROCEEDINGS

VOLUME 1

BALTIMORE, MD.
1942

QH 301
F29
v.1

Prog

Abst
Abst
Abst
Abst
Abst
Inde
Inde

Abst
Note
Meta
Nutr
New
Amir
Signi
Some
Phys
The
The
The

The
Tran
The
The
Some
Food
The
Vitar
Vitar
Vitar
Defir
Food
Clini
Guid
The

Anno
Fede
Exec
Form
By-L
Place
The
The
The

CONTENTS

No. 1, Part I, March 1942

Program of Twenty-ninth Annual Meeting, Boston 1

Part II

Abstracts of papers in Physiology 1
Abstracts of papers in Biochemistry 97
Abstracts of papers in Pharmacology 141
Abstracts of papers in Pathology 173
Abstracts of papers in Nutrition 187
Index of Authors 193
Index of Subjects 196

No. 2, June 1942

Abstracts, Corrections of Errors in 201
Notes on Boston Meeting 201
Metabolism of Estrogens 202
Nutritional Problems in Relation to the Nation's Health 209
Newer Viewpoints on Clinical Anesthesia 213
Amino Acids in Hemoglobin Formation 219
Significance of the Essential Fatty Acids 224
Some Morphological Aspects of the Secretory Process 233
Physicochemical Aspects of the Secretory Process 240
The Secretion of Dyestuffs by the Gastric Glands and Pancreas 246
The Formation of Acid by the Gastric Glands 255
The Effect of Nervous and Hormonal Stimulation on Zymogen Secretion 261

No. 3, September 1942

The Amino Acid Requirements and Protein Metabolism of the Avian Organism 269
Transamination 273
The Metabolism of the *d*-Amino Acids 281
The Relation of Vitamin C to the Metabolism of the Aromatic Amino Acids 287
Some Specific Physiological Disturbances Induced by Marginal Vitamin Deficiencies (C and B_1) 293
Foods as Sources of the Vitamins 296
The Biological Action of the Vitamins 304
Vitamins as Pharmacological Agents 309
Vitamins in the Practice of Medicine 316
Vitamins and Public Health 319
Definitions and Standards of Identity for Food 324
Food Consumption Studies and Dietary Recommendations 327
Clinical Observations Bearing on Food Requirements 330
Guiding Principles for the Fortification of Foods 336
The Laboratory Control of Enriched Foods 343

No. 4, December 1942

Announcement of Cancellation of the Cleveland Meeting 353
Federation of American Societies for Experimental Biology 353
Executive Committee, 1942 353
Former Executive Committees 353
By-Laws of the Federation 356
Placement Service 357
The American Physiological Society, 1942 357
The American Society of Biological Chemists, 1942 360
The American Society for Pharmacology and Experimental Therapeutics, 1942 365

806358

The American Society for Experimental Pathology, 1942 368
The American Institute of Nutrition, 1942 370
The American Association of Immunologists, 1942 373
Membership List of All Societies, 1942 374
Summary of Membership, 1942 438
Members Deceased, 1942 438
Index 441

FEDERATION OF AMERICAN SOCIETIES FOR EXPERIMENTAL BIOLOGY

CONTENTS

Adrenals 7
Analgesics 22
Anesthetics and hypnotics 24
Autonomic drugs 23
Biochemistry program 16
Blood 4
Blood flow—blood pressure 11
Brain 7
Brain potentials 3
Cardiac glycosides 25
Cardiodynamics 3
Chemotherapy, amines, circulatory drugs 25
Deficiency diseases 26
Demonstrations 2
Diabetes and allied problems 9
Electrolytes 20
Electrophysiology 3
Endocrines 5
Enzymes 21
Fats 18
Gastro-intestinal physiology 10
Hearing 11
Heart 6
Hematology 30
Hormones 17
Hormones, excretion, convulsants 26
Kidney and circulatory system 31
Liver—water balance 6
Metabolism 6
Metabolism, carbohydrate 16, 19
Metabolism, cellular 4
Metabolism, nitrogen 16, 18
Motion pictures 2
Muscle physiology 12
Neoplasms and hyperplasia 31
Neurophysiology 5
Nutrition program 32
Opiates 22
Pathology program 29
Pharmacology program 22
Phospholipids 18
Physiology program 2
Proteins 18, 21
Respiration 9
Sex hormones 13
Shock and allied problems 8
Steroids 17
Sulfonamides 27
Symposia:
Conference on statistical problems 20
Contributions of physics to biochemistry 18
Mechanism of secretion 13
Metabolism of protein 17
Morphine problems 23
Vitamins and enzyme action 16
Toxicity and tolerance 24
Vision 11
Vitamins 20
Vitamins: microbiology 29

GENERAL INFORMATION

Each of the constituent societies will have its own headquarters, as follows:

Physiology ..... Hotel Statler
Biochemistry ..... The Copley-Plaza
Pharmacology ..... The Brunswick
Pathology ..... The Parker House
Nutrition ..... Hotel Lenox

As far as possible all scientific sessions of each society will be held in its own headquarters. The Federation Joint Session and the Annual Smoker will be held in the Ball Room of the Hotel Statler. Registration and Static Demonstrations will be on the mezzanine of The Hotel Statler. Motion picture demonstrations will be shown in the salon of The Copley-Plaza. No annual dinner will be held.

Smoker. The Local Committee will provide an informal Smoker Thursday evening at 8:30 o'clock. Tickets, 50 cents each, may be secured at the registration desk. Refreshments, entertainment and music for dancing will be provided.

Registration will start Tuesday, March 31, at 10 a.m. at the *Hotel Statler*. A registration fee of one dollar will be required from each person in attendance, members as well as non-members. The registration fee does not include copies of the program (price, 50 cents) or the abstracts of papers (price, $1.50) which may be purchased at the registration desk. Information regarding points of interest in Boston, schedules of sightseeing trips, maps, etc., will be available.

Note. Since publication of the Local Committee announcement in the Federation Yearbook the price of all Boston Hotel rooms has been increased 10%.

Transportation between Hotels. Adequate taxi-cab service will be available in cabs marked "Federation." The fare, from the Hotel Statler to any other headquarters hotel, or return, will be ten cents per person, provided there are three or more passengers per cab.

No person will be admitted to any of the scientific sessions or demonstrations who cannot show the official registration badge.

The Women's Committee will have a desk adjoining the registration desk where wives of members and associate members may register without fee and obtain information regarding special arrangements planned for their entertainment.

Physiology Editorial Conference. Wednesday, 8:00 p.m. Parlor B, Hotel Statler. Those interested in the problems of scientific publication are invited to attend.

Placement Service. The Placement Service of the Federation, Dr. H. B. Lewis, Director, purposes without charge to facilitate communication between individuals seeking teaching and research positions in the biological sciences and institutions having vacancies. The Service is not limited to members of the Federation.

Dr. Lewis will be available for consultation in Parlor A of The Copley-Plaza Hotel:

Wednesday, 3:30–5:30 p.m.
Thursday, 3:30–5:30 p.m.
Friday, 3:30–5:30 p.m.

Appointments to see Dr. Lewis may be made in the same room on Wednesday between 2:30 and 3:30 and on Thursday between 11:00 and 12:00.

Business Meetings:

Physiology, Georgian Room, Hotel Statler, Thursday, 4:30 p.m.; Friday, 11:30 a.m.
Biochemistry, Foyer, The Copley-Plaza, Thursday, 5:00 p.m.; Friday, 11:30 a.m.
Pharmacology, Ball Room, The Brunswick, Thursday, 4:30 p.m.; Friday, 11:30 a.m.
Pathology, Hawthorne Room, The Parker House, Thursday, 5:00 p.m.
Nutrition, Ball Room, The Copley-Plaza, Wednesday, 11:00 a.m. and 5:00 p.m.

B & Z Library

FEDERATION PROGRAM

JOINT SESSION OF THE FEDERATION

Thursday, 10:00 a.m.

BALL ROOM, HOTEL STATLER

1. **Edward A. Doisy,** *St. Louis University*
   Metabolism of estrogens.
2. **John R. Murlin,** *University of Rochester*
   Nutritional problems in relation to the nation's health.
3. **Ralph M. Waters,** *University of Wisconsin*
   Newer viewpoints on clinical anesthesia.
4. **F. R. Robscheit-Robbins,** *University of Rochester*
   Amino acids in hemoglobin formation.
5. **George O. Burr,** *University of Minnesota*
   Significance of the essential fatty acids.

MOTION PICTURES

SALON, THE COPLEY-PLAZA

Thursday and Friday

1. **Robert Chambers and B. W. Zweifach,** *New York University*
   Caliber changes of the vessels of the capillary bed.
2. **Martin B. Macht** (*by invitation*) **and Philip Bard,** *Johns Hopkins University*
   Studies on decerebrate cats in the chronic state.
3. **Clinton N. Woolsey, George J. Hayes** (*by invitation*) **and Stanton E. Deeley** (*by invitation*), *Johns Hopkins University*
   Fibrillation in the unilaterally denervated diaphragm of the rat.

STATIC DEMONSTRATIONS

MEZZANINE, HOTEL STATLER

Daily, 9:00 a.m. to 5:00 p.m.

1. **Georg Barkan,** *Boston University School of Medicine*
   Secondary storage of thorium in the bone-marrow of rabbits.
2. **Howard H. Beard and Walter B. Coffey** (*by invitation*), *Louisiana State University and the Southern Pacific General Hospital, San Francisco.* With the assistance of Harry Coffey, Julia K. Espenen and Phillip Pizzolato
   Experimental and clinical observations on the effect of adrenal extracts, vitamins, amino acids, hormones, etc., upon the appearance, growth and regression of transplantable tumors in rodents and inoperable tumors in man.
3. **H. S. Bennett and W. H. Sweet,** *Harvard Medical School*
   A heated thermocouple flow-meter for recording flow in blood vessels of experimental animals.
4. **Wilhelm Buschke, Anthony A. Albanese and Richard H. Follis, Jr.** (*introduced by* Edwards A. Park), *Johns Hopkins University*
   Pathological changes in tryptophane deficiency.
5. **Sergei Feitelberg** (*introduced by E. P. Pick*), *Mount Sinai Hospital, New York, N. Y.*
   A photoelectrical recorder for biological purposes.
6. **O. H. Gaebler,** *Henry Ford Hospital, Detroit*
   Studies of the nitrogen storage produced by anterior pituitary preparations.
7. **Robert Gesell, Charles R. Brassfield, Elwood T. Hansen and Arnold Mason** (*by invitation*), *University of Michigan*
   An acid-neurohumoral mechanism of gradation of stimulation. Demonstration of the principles on heart, striated muscle and intestine and a summary of experimental findings with original tracings.
8. **Harold D. Green,** *Western Reserve University*
   A glass membrane optical manometer.
9. **Harold D. Green, Richard S. Cosby** (*by invitation*), **Karl H. Radzow** (*by invitation*), *Western Reserve University*
   Evaluation of the collateral circulation artifacts which complicate studies of regional vascular reactions.
10. **D. E. Gregg, R. E. Shipley** (*by invitation*), **W. H. Pritchard** (*by invitation*), **R. W. Eckstein** (*by invitation*), **J. Dingle Kent** (*by invitation*) **and J. T. Wearn,** *Western Reserve University*
    Some errors in the application of the thermostromuhr method.
11. **F. A. Hellebrandt, E. Corinne Fries** (*by invitation*) **and Eleanor M. Larsen** (*by invitation*), *University of Wisconsin*
    The influence of the regulation army pack on body alignment and postural stability.
12. **L. Emmett Holt, Jr. and Victor A. Najjar,** *Johns Hopkins University*
    A novel method of presenting scientific papers.
13. **Brenton R. Lutz, George P. Fulton** (*by invitation*) **and Jack I. Seltzer** (*by invitation*), *Boston University*
    Sphincter mechanisms, smooth muscle motor-units and vascular patterns in the blood vessels of the retrolingual membrane of Rana pipiens.

14. **Walter L. Mendenhall,** *Boston University School of Medicine*
New methods in experimental pharmacology.
15. **V. A. Najjar and L. Emmett Holt, Jr.,** *Johns Hopkins University*
A fluocomparator for quantitative fluorescence measurements.
16. **Elaine P. Ralli and Irving Graef,** *New York University College of Medicine*
The influence of adrenalectomy in black rats on diets deficient and adequate in the filtrate fractions of vitamin B.
17. **Warren M. Sperry,** *New York State Psychiatric Institute*
A procedure for the electrophotometric microdetermination of phosphorus in lipid extracts.
18. **F. William Sunderman,** *University of Pennsylvania*
Estimation of total base in serum by means of conductivity and specific gravity measurements.
19. **Carl Voegtlin,** *National Cancer Institute*
Experimental tumors of the liver.
20. **J. H. Weatherby,** *Medical College of Virginia*
Concerning the mechanism of membrane semipermeability.
21. **Herbert S. Wells,** *Wake Forest College*
A portable apparatus for measuring blood flow in the extremities.

PHYSIOLOGY A

Thursday 2:00 p.m.

BALL ROOM ASSEMBLY

Electrophysiology—Brain Potentials

1. **W. S. Rehm** (*introduced by* Hampden Lawson), *University of Louisville*
The effect of variations in external resistance on the electrical activity of the stomach.
2. **Kenneth S. Cole and George Marmont,** *Columbia University*
The effect of ionic environment upon the longitudinal impedance of the squid giant axon.
3. **Judith Graham** (*by invitation*), **G. R. Carlson** (*by invitation*) **and R. W. Gerard,** *University of Chicago*
Membrane and injury potentials of single muscle fibers.
4. **R. W. Gerard and Judith Graham** (*by invitation*), *University of Chicago*
Excitation and membrane potentials of single muscle fibers.
5. **J. M. Snodgrass** (*introduced by* Birdsey Renshaw), *Oberlin College*
Simple electric integration of action potentials as a direct index of muscular activity.
6. **J. E. P. Toman** (*by invitation*) **and Robert H. Oster,** *University of Maryland*
The human electromyogram in brief volitional twitches.
7. **Morton A. Rubin** (*by invitation*), **Hebbel E. Hoff, Alexander W. Winkler and Paul K. Smith** (*by invitation*), *Worcester State Hospital and Yale University*
The influence of intravenous potassium, calcium and magnesium on the electrocorticogram of the cat.
8. **M. Kessler** (*by invitation*), **H. Hailman** (*by invitation*) **and E. Gellhorn,** *University of Illinois*
Influence of lowered barometric pressure on the E.E.G. under various conditions.
9. **F. A. Gibbs, W. G. Lenox** (*by invitation*), **L. F. Nims and E. L. Gibbs** (*by invitation*), *Harvard Medical School*
Differentiation of the effect of low O_2 and low CO_2 on the electrical activity of the cortex.
10. **Chester W. Darrow,** *University of Illinois*
Excitatory and homeostatic effects on the electroencephalogram.
11. **B. L. Pacella** (*by invitation*), **S. E. Barrera and L. M. Kopeloff** (*by invitation*), *N. Y. State Psychiat. Inst. and Hosp.*
Electroencephalographic studies on monkeys with chronic Jacksonian seizures.
12. **Paul F. A. Hoefer** (*introduced by* F. A. Mettler) **and J. Lawrence Pool** (*introduced by* F. A. Mettler), *Columbia University*
The conduction of cortical impulses and the motor management of convulsive seizures.

PHYSIOLOGY B

Thursday, 2:00 p.m.

GEORGIAN ROOM

Cardiodynamics

1. **K. Jochim** (*introduced by* L. N. Katz), *Michael Reese Hospital*
Further observations on the dynamics of fluid flow in an elastic system.
2. **Philip Dow and W. F. Hamilton,** *University of Georgia*
Further ballistic studies on cardio-aortic hydraulic models.
3. **W. F. Hamilton and Philip Dow,** *University of Georgia*
The recoil curves of the circulation in three dimensions.
4. **Robert H. Shuler** (*by invitation*), **Charles Ensor** (*by invitation*), **R. E. Gunning** (*by*

invitation), **W. G. Moss** (*by invitation*) **and Victor Johnson,** *University of Chicago*
The differential effects of respiration on the left and right ventricles.

5. **T. E. Boyd and Mary C. Patras** (*by invitation*), *Loyola University*
Circulatory effects of brief passive inflation and deflation of the lungs.
6. **J. P. Holt** (*introduced by* H. Lawson), *University of Louisville*
The effect of positive and negative intrathoracic pressure on cardiac output in the dog.
7. **J. F. Herrick, C. F. Code, A. R. Maclean** (*by invitation*) **and F. C. Mann,** *Mayo Foundation*
Vascular changes associated with short periods of raised intrapulmonary pressures.
8. **H. A. Blair, A. M. Wedd** (*by invitation*) **and H. M. Hardwicke** (*by invitation*), *University of Rochester*
The normal pneumocardiogram.
9. **E. H. Wood and G. K. Moe** (*introduced by* M. B. Visscher), *University of Minnesota*
Effects of elevated serum potassium levels on the heart-lung preparation.
10. **F. A. Hellebrandt and Percy M. Dawson,** *University of Wisconsin*
The influence of ageing on the cardiovascular response to exercise.

PHYSIOLOGY C

Thursday, 2:00 p.m.

PARLOR A

Blood

1. **Barry G. King, Enid T. Oppenheimer and Kenneth S. Cole,** *Columbia University*
The disappearance of the dye T-1824 from the blood stream.
2. **Minerva Morse, Frederic W. Schlutz and Donald E. Cassels** (*by invitation*), *University of Chicago* (Biochem.)
Blood, plasma and "available fluid" volumes of boys 10–16 years of age. (Biochem.)
3. **Q. B. DeMarsh** (*by invitation*), **W. F. Windle and H. L. Alt** (*by invitation*), *Northwestern University*
Blood volume of the newborn in relation to early and late clamping of the umbilical cord.
4. **William R. Amberson, Joye E. Jacobs** (*by invitation*) **and Alan Hisey** (*by invitation*), *University of Maryland and George Washington University*
Human hemoglobin solutions as a blood substitute.
5. **Joye E. Jacobs** (*by invitation*), **Alan Hisey** (*by invitation*) **and W. R. Amberson,** *University of Maryland and George Washington University*
Preservation of human hemoglobin solutions without methemoglobin formation.
6. **N. A. Masor** (*by invitation*), **Harry Greengard, W. L. Voegtlin** (*by invitation*), **J. P. Sandblom** (*by invitation*) **and A. C. Ivy,** *Northwestern University*
Intercompatible reactions to blood transfusion related to the donor's diet.
7. **G. Miley** (*introduced by* R. Beutner), *Hahnemann Medical College*
The ultraviolet irradiation of auto-transfused blood: Studies in combined oxygen values.
8. **L. W. Freeman** (*by invitation*), **A. Loewy** (*by invitation*), **A. Marchello** (*by invitation*), **and Victor Johnson,** *University of Chicago*
Increased total bile pigment output on a high fat diet.
9. **Joan Longini** (*by invitation*), **L. W. Freeman** (*by invitation*) **and Victor Johnson,** *University of Chicago*
Increased red blood cell fragility during lipemia.

PHYSIOLOGY D

Thursday 2:00 p.m.

PARLORS B AND C

Cellular Metabolism

1. **Frederick A. Fuhrman** (*by invitation*) **and John Field 2nd,** *Stanford University*
Influence of temperature on the stimulation of oxygen consumption of isolated rat brain cortex by 2–4 dinitrophenol.
2. **Arthur W. Martin** (*by invitation*), **Frederick A. Fuhrman** (*by invitation*) **and John Field 2nd,** *Stanford University and University of Washington*
Influence of temperature on respiration and glycolysis in isolated rabbit brain.
3. **Kenneth C. Fisher,** *University of Toronto*
An analysis of the oxygen consumption of yeast as related to cell division.
4. **Attilio Canzanelli and David Rapport,** *Tufts College*
Deamination of d-l alanine by kidney slices following administration of growth hormone complex.
5. **P. W. Davies and Frank Brink, Jr.** (*introduced by* D. W. Bronk), *University of Penna.*
Direct measurement of brain oxygen concentrations with a platinum electrode.
6. **Joseph Wortis** (*introduced by* I. M. Korr), *New York University*

The effect of serum on the survival and revival of brain oxidations.

7. **J. M. Tobias** (*by invitation*), **D. B. Clark** (*by invitation*) **and R. W. Gerard,** *University of Chicago*
   Spinal ganglia respiration.
8. **A. Van Harreveld and D. B. Tyler** (*by invitation*), *California Institute of Tech.*
   The respiration of the spinal cord following 60 minutes of asphyxiation.
9. **Harold E. Himwich, Herman Herrlich** (*by invitation*), **Edith Rich** (*by invitation*), **Robert Barstow** (*by invitation*) **and Joseph F. Fazekas** (*by invitation*), *Albany Medical College*
   Effect of iodoacetate on survival period during hypoxia.

PHYSIOLOGY E

Thursday 2:00 p.m.

Salle Moderne

Endocrines

1. **Boris B. Rubenstein,** *Michael Reese Hospital*
   Human sperm survival in vivo.
2. **William W. Scott** (*by invitation*), **Charles Huggins and J. Henry Heinen** (*by invitation*), *University of Chicago*
   Chemical composition of human prostatic and vesicular fluids and of semen.
3. **Savino A. D'Angelo** (*by invitation*), **Albert S. Gordon** (*by invitation*) **and Harry A. Charipper,** *New York University*
   Thyrotropic hormone assay in the tadpole.
4. **Allen Lein** (*introduced by* George M. Curtis), *Ohio State University*
   Studies on the activity of the goitrous thyroid gland.
5. **W. Fleischmann, H. B. Shumacker, Jr.** (*by invitation*) **and W. L. Straus, Jr.** (*by invitation*), *Johns Hopkins University*
   The absence of effect of thyroidectomy on the adult Rhesus monkey.
6. **D. S. Riggs** (*introduced by* W. T. Salter) **and E. B. Man** (*by invitation*), *Yale University*
   The effect of environmental temperature on thyroid function in dogs.
7. **Harold N. Ets and R. D. Templeton,** *Loyola University*
   The effect of thyroid parathyroidectomy on the cold blocking temperature of rat nerves.
8. **Karl E. Paschkis** (*introduced by J. Earl Thomas*), *Jefferson Medical College*
   Action of anterior pituitary growth hormone on protein metabolism of rats poisoned with monoiodoacetic acid.
9. **R. O. Greep, Bacon F. Chow and H. B. Van Dyke,** *Squibb Institute for Medical Research*
   The action of lactogenic hormone on tissues other than the mammary gland.
10. **Robert Gaunt, E. C. Kendall and W. J. Eversole** (*by invitation*), *Mayo Foundation*
    Maintenance of lactation in adrenalectomized rats.
11. **W. E. Petersen and T. M. Ludwick** (*introduced by* M. B. Visscher), *University of Minn.*
    The hormoral nature of the factor causing the letdown of milk.

PHYSIOLOGY A

Friday 9:00 a.m.

Ball Room Assembly

Neurophysiology

1. **G. H. Atcheson and C. D. Ratnoff** (*introduced by* A. Rosenblueth), *Harvard Medical School*
   The localized effect on the spinal cord of intramuscularly injected tetanus toxin.
2. **Birdsey Renshaw,** *Rockefeller Institute and Oberlin College*
   Excitation of spinal neurons by centripetal impulses in ventral root axons.
3. **David P. C. Lloyd,** *Rockefeller Institute*
   Activity in pathways concerned with long spinal reflexes.
4. **R. F. Pitts,** *New York University Medical College*
   The site of mechanisms determining frequency and recruitment of phrenic neurones.
5. **Amedeo S. Marrazzi** (*introduced by* G. B. Wallace), *New York University Medical College*
   The distribution and nature of ganglionic inhibition in the sympathetic system.
6. **D. Nachmansohn,** *Yale University*
   Electrical potential and activity of cholinesterase in nerve.
7. **E. T. Hansen** (*by invitation*), **J. J. Worzniak** (*by invitation*) **and Robert Gesell,** *University of Michigan*
   Physiological effects of artificially administered acetylcholine and eserine.
8. **Charles R. Brassfield and Robert Gesell,** *University of Michigan*
   Examples of the acid-neurohumoral mechanism of nervous integration.
9. **Arnold Mason** (*by invitation*) **and Robert Gesell,** *University of Michigan*
   Extra and intracellular cH and the stimulating action of acetylcholine.

10. **Robert Gesell, Charles R. Brassfield and Elwood T. Hansen** (*by invitation*), *University of Michigan*
Possible roles of cH in neurophysiology.

PHYSIOLOGY B

Friday 9:00 a.m.

GEORGIAN ROOM

Heart

1. **L. H. Nahum, H. E. Hoff and W. Kaufman** (*by invitation*), *Yale University*
The nature of the S wave.
2. **W. Kaufman** (*by invitation*), **L. H. Nahum and H. E. Hoff,** *Yale University*
Distribution in leads I and III of potentials applied to the surface of the heart.
3. **Emil Bozler,** *Ohio State University*
Local potentials and tonus changes in cardiac muscle and their relation to the initiation of impulses.
4. **Alberto Guevara Rojas** (*by invitation*), **A. Sidney Harris and Demetrio Sodi Pallares** (*by invitation*), *Western Reserve University*
Thresholds of ventricular fibrillation by galvanic currents. Effects of barbiturates and of citrate.
5. **A. Sidney Harris, Alberto Guevara Rojas** (*by invitation*) **and Detrio Sodi Pallares** (*by invitation*), *Western Reserve University*
Fibrillation and other functional changes in mammalian ventricles resulting from coronary occlusion.
6. **René Wégria and Neil Nickerson** (*by invitation*), *Western Reserve University*
Quinidine sulfate and ventricular fibrillation.
7. **Warren E. Gilson** (*by invitation*), **Ruth E. Conklin** (*by invitation*) **and J. A. E. Eyster,** *University of Wisconsin*
The relation of fractionate contraction at various surface regions of the ventricle of the snapping turtle to the surface potential distribution.
8. **J. A. E. Eyster, Walter J. Meek, Ruth E. Conklin** (*by invitation*) **and Warren E. Gilson** (*by invitation*), *University of Wisconsin*
The relation of fractionate contraction in regions of the right auricle of the dog's heart to the surface potential distribution.
9. **Robert F. Furchgott** (*by invitation*) **and Ephraim Shorr** (*with the technical assistance of* Gladys Brewer), *New York University and Cornell Medical School*
Phosphate exchange in resting cardiac muscle as indicated by radio-activity studies: II.
10. **W. V. Whitehorn** (*by invitation*) **and J. W. Bean,** *University of Michigan*
Causal mechanisms in the cardiac effects of high oxygen pressures.

PHYSIOLOGY C

Friday 9:00 a.m.

PARLOR A

Liver-Water Balance

1. **Adrian Borduas** (*by invitation*), **Christiane Dosne** (*by invitation*) **and Hans Selye,** *McGill University*
Role of the liver in experimental uremia.
2. **Henry N. Harkins, Brock Brush, R. T. Boals and C. Frank Chunn** (*introduced by* L. R. Dragstedt), *Henry Ford Hospital*
The role of the liver in alimentary axotemia.
3. **Victor H. Hough and Smith Freeman** (*by invitation*), *Northwestern University*
The effect of a protein-deficient diet upon the serum phosphatase and hepatic dye clearance of dogs.
4. **Lathan A. Crandall, Jr. and Alys Lipscomb,** *University of Tennessee*
Hepatic blood flow and glucose output in unanesthetized dogs.
5. **Oscar Bodansky and Walter Modell** (*by invitation*), *Cornell Medical School*
The fecal and urinary excretion, the tissue distribution and the serum concentration of bromide and chloride during prolonged bromide administration. (Biochem.)
6. **Everett G. Weir,** *Howard University*
Observations on the distribution of bromide between cerebro-spinal fluid and serum.
7. **Arnoldus Goudsmit and Lawrence Louis** (*by invitation*), *University of Pennsylvania*
Extracellular fluid volume in man, and its relation to season.
8. **E. F. Adolph,** *Rochester Medical School*
Diuresis and the recovery of water balance.
9. **A. V. Wolf** (*introduced by* E. F. Adolph), *University of Rochester*
The relative retentions of infused chloride, urea and water.
10. **Otis M. Cope and Frederick M. Allen,** *New York Medical College*
Some effects of salt and water ingestion.

PHYSIOLOGY D

Friday 9:00 a.m.

PARLORS B AND C

Metabolism

1. **F. R. Steggerda,** *University of Illinois*
Observations on the concentration and absorption of minerals by the rectal mucosa.

2. **H. F. Blum, H. G. Grady** (*by invitation*) **and J. F. Kirby-Smith** (*by invitation*), *National Cancer Institute*
   The effect of ultraviolet radiation on body weight of mice.
3. **Nathan W. Shock** (*introduced by* Harold F. Blum), *University of California and National Institute of Health*
   The effect of menarche on basal physiological functions in girls.
4. **Peter V. Karpovich and Nathan Millman** (*introduced by* W. B. Cannon), *Springfield College*
   Energy expenditure in swimming.
5. **Broda Barnes,** *University of Denver*
   Basal metabolism in acne vulgaris.
6. **Sid Robinson,** *Indiana University*
   The effect of body size upon energy exchange in work.
7. **Eugene S. Turrell** (*introduced by* Sid Robinson), *Indiana University*
   The acid-base equilibrium of the blood in exercise.
8. **Jay Tepperman** (*by invitation*), **J. R. Brobeck** (*by invitation*) **and C. N. H. Long,** *Yale University*
   The effect of changes in food habit upon the respiratory exchange of intact and eviscerate rats.
9. **S. B. Barker, J. A. Dye and W. B. Chambers,** *Cornell Medical School*
   Fructose metabolism in the fasting dog.
10. **Walter Goldfarb and Ernest Beuding,** *Bellevue Hospital and New York University*
    Blood changes following glucose, lactate and pyruvate injections in man.

PHYSIOLOGY E

Friday 9:00 a.m.

Salle Moderne

Adrenals

1. **S. Rodbard** (*introduced by* L. N. Katz), *Michael Reese Hospital*
   Retardation of uremic death by desoxycorticosterone acetate.
2. **Alexander W. Winkler, Paul K. Smith** (*by invitation*) **and Hebbel E. Hoff,** *Yale University*
   Spontaneous potassium poisoning in the adrenalectomized animal.
3. **A. Roos** (*introduced by* E. B. Astwood), *Harvard Medical School and Peter Bent Brigham Hospital*
   The assay of adrenal cortical extract in adrenalectomized rats exposed to cold.
4. **Richard Tyslowitz** (*by invitation*) **and E. B. Astwood,** *Harvard Medical School and Peter Bent Brigham Hospital*
   The influence of hypophysectomy on the size, function, and ascorbic acid content of the adrenal gland of the rat.
5. **E. B. Astwood and R. Tyslowitz** (*by invitation*), *Harvard Medical School and Peter Bent Brigham Hospital*
   An assay method for corticotrophin.
6. **Christiane Dosne** (*by invitation*) **and Hans Selye,** *McGill University*
   The physiological significance of compensatory adrenal atrophy.
7. **Elaine P. Ralli and Irving Graef,** *New York University*
   The influence of adrenalectomy in black rats on normal and deficient diets, with special reference to pigmentation of the coat and hair follicles.
8. **Caroline Tum-Suden and Leland C. Wyman,** *Boston Medical School and Mass. Mem. Hosp.*
   The hemodiluting capacity of rats with adrenocortical transplants.
9. **Gordon C. Ring,** *Ohio State University*
   The importance of the adrenal medulla during prolonged exposure to cold.
10. **Harry B. Friedgood,** *Peter Bent Brigham Hospital and Harvard Medical School*
    On the physiological significance of the abnormally decreased or absent 17-ketosteroid excretion in Addison's disease, panhypopituitarism and myxedema.

PHYSIOLOGY A

Friday 2:00 p.m.

Ball Room Assembly

Brain

1. **S. C. Wang** (*introduced by* Magnus I. Gregersen), *Columbia University*
   Localization of the salivatory "points" in the lower brain stem of cat.
2. **J. R. Blair** (*by invitation*) **and A. D. Keller,** *University of Alabama*
   Observations pertaining to the localization of nerve fiber pathways subserving heat regulating functions in the midbrain and pons.
3. **Harry Grundfest and Berry Campbell** (*by invitation*), *Rockefeller Institute*
   Activity in the dorsal spino-cerebellar tract and its effect in the cerebellum.

4. **Ray S. Snider and Averill Stowell** (*introduced by* Philip Bard), *Johns Hopkins University*
   Evidence of a representation of tactile sensibility in the cerebellum of the cat.
5. **Averill Stowell** and **Ray S. Snider** (*introduced by* Philip Bard), *Johns Hopkins University*
   Evidence of a representation of auditory sensibility in the cerebellum of the cat.
6. **William F. Allen,** *University of Oregon*
   Cortical irradiation in conditioned differentiation as measured by effects of respiration.
7. **Edward Girden** (*introduced by* E. A. Culler), *Brooklyn College*
   Striate and autonomic components of conditioned reflexes established under erythroidine and curare.
8. **Simon Dworkin, Judith O. Baxt** (*by invitation*) **and J. Gross** (*by invitation*), *McGill University*
   Deafness "neurosis" in the cat as a special form of disinhibition.
9. **Victor H. Rosen** (*by invitation*) **and W. Horsley Gantt,** *Johns Hopkins University*
   The effect of metrazol convulsions on conditioned reflex training in dogs.
10. **W. Horsley Gantt,** *Johns Hopkins University*
    Cardiac conditioned reflexes to painful stimuli.
11. **J. R. Brobeck** (*by invitation*), **Jay Tepperman** (*by invitation*) **and C. N. H. Long,** *Yale University*
    Further observations on experimental hypothalamic obesity.
12. **Chandler McC. Brooks, E. F. Lambert** (*by invitation*) **and Philip Bard,** *Johns Hopkins University*
    Experimental production of obesity in the monkey (Macaca mulatta).
13. **T. C. Ruch, H. D. Patton** (*by invitation*) **and J. R. Brobeck** (*by invitation*), *Yale University*
    Hyperphagia and adiposity in relation to disturbances of taste.
14. **V. Arnett** (*by invitation*) **and E. Gellhorn,** *University of Illinois*
    Changes in the autonomic centers after repeated electrically induced convulsions.
15. **E. Spiegel, M. Spiegel-Adolf** (*by invitation*) **and G. Henny** (*by invitation*), *McCarthy Foundation and Temple University*
    Changes of conductance and of cellular permeability induced by electrical stimulation of the brain.
16. **M. J. Oppenheimer** (*by invitation*), **E. A. Spiegel, K. Weston** (*by invitation*), *Temple University*
    Cortical pre- and postmotor foci influencing the gastro-intestinal tract and their efferent pathways.

PHYSIOLOGY B

Friday 2:00 p.m.

GEORGIAN ROOM

Shock and Allied Problems

1. **H. S. Mayerson,** *Tulane University*
   Studies on gravity shock in the dog.
2. **F. J. Manery, D. Y. Solandt and E. Randell** (*by invitation*), *University of Toronto*
   Further studies on shock with particular reference to electrolytes.
3. **A. E. Schecter** (*by invitation*), **M. L. Cullen** (*by invitation*) **and N. E. Freeman,** *University of Pennsylvania*
   The production of traumatic shock in dogs after total sympathectomy and after spinal cord transection.
4. **Harold C. Wiggers, G. H. Glaser** (*by invitation*), **K. deS. Canavarro** (*by invitation*) **and A. E. Treat** (*by invitation*), *Columbia University*
   The effect of gross bilateral lesions of the spinal cord (C-8 to T-1) on the effective venous pressure in the anesthetized cat.
5. **Harold Lamport** (*introduced by* Fred A. Mettler), *Columbia University*
   Charts for osmotic pressure of blood. Application of formulas for renal arteriolar resistance.
6. **J. R. Pappenheimer and J. P. Maes** (*introduced by* M. I. Gregersen), *Columbia University*
   The effects of vasoconstriction on the apparent viscosity of blood flowing through the hindlimb of the dog.
7. **J. P. Maes and J. R. Pappenheimer** (*introduced by* Magnus I. Gregersen), *Columbia University*
   Factors affecting the vasomotor tone in the hindlimb muscles of the dog.
8. **Harold D. Green and** (*by invitation*) **Richard S. Cosby and Robert N. Lewis,** *Western Reserve University*
   Modification of the blood flow in skin and muscle of dogs in hemorrhagic shock.
9. **C. J. Wiggers and** (*by invitation*) **Jacob M. Werle and P. C. Shea,** *Western Reserve University*
   Cardiac and peripheral determinants of hemorrhagic shock.
10. **Wm. H. Olson and H. Necheles,** *Michael Reese Hospital*
    A blood pressure raising principle in thermal shock.
11. **Dean A. Collins and Angie S. Hamilton** (*by invitation*), *Temple University*

Role of a renal humoral mechanism in hemorrhage.

12. **L. N. Katz and** (*by invitation*) **S. Killian, S. Perlow and R. Asher,** *Michael Reese Hospital*
The effect of desoxycorticosterone acetate in shock due to massive venous thrombosis of a leg. An experimental study.
13. **Angie S. Hamilton** (*by invitation*) **and Dean A. A. Collins,** *Temple University*
Role of a renal humoral mechanism in shock.
14. **J. M. Rogoff and E. Nola Nixon** (*by invitation*), *University of Pittsburgh*
Blood pressure studies on dogs.
15. **Henry F. Helmholz, Jr.** (*introduced by* F. C. Mann), *Mayo Foundation*
The response of the blood pressure and pulse rate of the newborn rat to changes in body temperature.
16. **R. E. Conklin and T. H. Simister** (*by invitation*), *Vassar College*
The role of pressoreceptors in the regulation of blood pressure in the rabbit.

PHYSIOLOGY C

Friday 2:00 p.m.

PARLOR A

Diabetes and Allied Problems

1. **Isabelle Grayman** (*by invitation*), **Norton Nelson** (*by invitation*), **Samuel Elgart** (*by invitation*) **and I. Arthur Mirsky,** *May Institute*
Pancreatic diabetes in the monkey.
2. **Samuel Elgart** (*by invitation*), **Norton Nelson** (*by invitation*) **and I. Arthur Mirsky,** *May Institute*
The production of permanent hyperglycemia and glycosuria by the prolonged administration of insulin.
3. **I. Arthur Mirsky, Norton Nelson** (*by invitation*) **and Samuel Elgart** (*by invitation*), *May Institute*
Pancreatic diabetes in the owl.
4. **D. R. Drury,** *University of Southern California*
The amount of glucose formed from protein in diabetics.
5. **Sidney Rosenberg** (*by invitation*) **and Leo T. Samuels,** *University of Minnesota*
The influence of previous diet on the insulin tolerance of hypophysectomized rats. (Pharmacol.)
6. **R. Levine** (*by invitation*), **R. N. Feinstein** (*by invitation*) **and Samuel Soskin,** *Michael Reese Hospital and University of Chicago*
Studies on the mechanism of insulin action in skeletal muscle in vitro.
7. **Jane A. Russell and Jay Tepperman,** *Yale University*
Carbohydrate metabolism in the eviscerated rat. II.
8. **Oscar Riddle and David F. Opdyke** (*by invitation*), *Carnegie Institution*
Gross differences in response of the liver to first and later daily dosage with insulin and prolactin.
9. **J. M. Bruhn, A. D. Keller, W. E. Lawrence** (*by invitation*), **and C. F. Barton** (*by invitation*), *University of Alabama*
Decrease in insulin tolerance following hypophysectomy largely due to removal of stalk tissue.
10. **A. D. Keller, J. M. Bruhn, C. F. Barton** (*by invitation*) **and W. E. Lawrence** (*by invitation*), *University of Alabama*
Pancreatectomy incompatible to life in the presence of near-total hypophysectomy in the dog.
11. **R. Ryer** (*introduced by* J. R. Murlin), *University of Rochester*
The effect of insulin, thyroxin and their combination on the energy metabolism of the rabbit.
12. **Saul H. Rubin** (*by invitation*) **and Elaine P. Ralli,** *New York University*
The influence of meat and meat fractions on the fatty liver of the depancreatized and pancreatic duct ligated dog.
13. **J. Garrott Allen** (*by invitation*), **Frederick M. Owens, Jr.** (*by invitation*), **Dorothy Stinger** (*by invitation*) **and Lester R. Dragstedt,** *University of Chicago*
The effect of partial pancreatectomy on the blood lipids.
14. **Frederick M. Owens, Jr.** (*by invitation*), **J. Garrott Allen** (*by invitation*), **Dorothy Stinger** (*by invitation*) **and Lester R. Dragstedt,** *University of Chicago*
The comparative effects of inositol and lipocaic in depancreatized dogs.
15. **Lester R. Dragstedt, J. Garrott Allen** (*by invitation*), **Frederick M. Owens, Jr.** (*by invitation*) **and G. Vermeulen** (*by invitation*), *University of Chicago*
Lipocaic and insulin sensitivity.

PHYSIOLOGY D

Friday 2:00 p.m.

PARLORS B AND C

Respiration

1. **Robert Hodes and M. G. Larrabee,** *University of Pennsylvania*

Single fiber analysis of inhibition of inspiration.

2. **J. H. Comroe, Jr.** (*by invitation*) **and Carl F. Schmidt,** *University of Pennsylvania*
Respiratory reflexes from the limbs.

3. **Claude V. Winder,** *University of Michigan*
Combination of hypoxic and hypercapnic stimulation at the carotid body.

4. **J. G. Watt, P. R. Dumke and J. H. Comroe, Jr.** (*introduced by* C. F. Schmidt), *University of Pennsylvania*
The part played by carotid and aortic body reflexes in respiratory control in unanesthetized dogs.

5. **Hayden C. Nicholson and Juay Hong** (*by invitation*), *University of Michigan*
Respiratory effects of brain stem transections.

6. **Juay Hong** (*by invitation*) **and Hayden C. Nicholson,** *University of Michigan*
Altered responses to various respiratory modifiers as a result of brain stem transections.

7. **F. G. Hall,** *Duke University*
A spectroscopic method for determintion of carbon monoxide in air.

8. **Chalmers L. Gemmill,** *Naval Air Station, Pensacola, Fla.*
The effects of lowered barometric pressure on man.

9. **Edward J. Van Liere and J. Clifford Stickney** (*by invitation*), *West Virginia Univ.*
Acclimatization during discontinuous exposure to anoxia.

10. **J. Clifford Stickney** (*by invitation*) **and Edward J. Van Liere,** *West Virginia Univ.*
Acclimatization to simulated altitudes in relation to gastric emptying.

11. **L. L. Langley** (*introduced by* J. F. Fulton), *Yale University*
The effect of exposure to low atmospheric pressure on the adrenal glands and on carbohydrate metabolism in the rat.

12. **Franklin F. Snyder,** *University of Chicago*
The oxygenation and carbon dioxide content of fetal blood during the interruption of fetal respiration coincident with the breathing of low oxygen mixtures by the maternal animal.

13. **R. F. Becker and W. F. Windle,** *Northwestern University*
The efficacy of O_2 and O_2-CO_2 mixtures for resuscitation of the newborn.

PHYSIOLOGY E

Friday 2:00 p.m.

SALLE MODERNE

Gastro-intestinal Physiology

1. **H. F. Adler and A. J. Atkinson** (*introduced by* A. C. Ivy), *Northwestern University*
Segmental activity of the colon.

2. **Stewart Wolf** (*by invitation*) **and Harold G. Wolff,** *New York Hospital and Cornell Medical College*
Studies on pain. Pain mechanisms in the stomach and observations on the sensibility of the gastric mucosa.

3. **T. L. Patterson and D. J. Sandweiss** (*by invitation*), *Wayne University and Harper Hospital*
The simultaneous gastric and duodenal motility phases in man.

4. **F. J. Ingelfinger and R. E. Moss** (*introduced by* F. H. Pratt), *Evans Memorial Hosp., Mass. Memorial Hosp. and Boston University*
The activity of the descending duodenum in man during nausea produced by caloric stimulation of the semi-circular canals.

5. **J. P. Quigley, M. R. Read** (*by invitation*), **K. H. Radzow** (*by invitation*), **I. Meschan** (*by invitation*) **and J. M. Werle** (*by invitation*), *Western Reserve University*
The influence of hydrochloric acid on the motor activities of the pyloric sphincter region and on the process of gastric evacuation.

6. **Edward E. Jemerin** (*by invitation*), **Franklin Hollander and Vernon A. Weinstein** (*by invitation*), *Mount Sinai Hospital* (*Biochem.*)
Criteria for differentiating vagal stomach pouches.

7. **Louis B. Dotti and Israel S. Kleiner,** *New York Medical College and Flower and Fifth Ave. Hosp.*
Is there rennin in human gastric juice?

8. **M. J. Schiffrin** (*by invitation*) **and John S. Gray,** *Northwestern University*
The effect of urogastrone on gastric secretion in enterectomized dogs.

9. **Jerzy Kaulbersz and James M. Winfield** (*introduced by* Charles G. Johnston), *Wayne University*
The influence of bile on gastric secretion.

10. **C. F. Code, G. A. Hallenbeck** (*by invitation*) **and R. A. Gregory** (*by invitation*), *Mayo Foundation*
The histamine content of gastric juice.

11. **Joseph E. Bourque** (*by invitation*) **and Earl R. Loew,** *Wayne University*
The effect of histamine antagonists on gastric secretion.
12. **H. Necheles, W. H. Olson and William Scruggs,** *Michael Reese Hospital*
The effect of insulin on gastric secretion.
13. **Robert L. Driver** (*introduced by* J. R. Murlin), *University of Kentucky*
Permeability as a factor in the etiology of ulcers.
14. **Raymond C. Herrin,** *University of Wisconsin*
The mechanism of azotemia of experimental intestinal obstruction.
15. **A. J. Kosman** (*by invitation*), **Jerzy W. Kaulbersz** (*by invitation*) **and Smith Freeman,** *Northwestern University*
The secretion of phosphatase by the intestine of the dog.
16. **Robert A. Roback** (*by invitation*), **Harry Greengard and A. C. Ivy,** *Northwestern University*
Sympathomimetic and related drugs stimulating pancreatic secretion.

PHYSIOLOGY A

Saturday 9:00 a.m.

Ball Room Assembly

Vision—Hearing

1. **Karl U. Smith** (*introduced by* W. K. Smith), *University of Rochester*
Monocular and binocular fusion in movement and depth perception after section of the commissures of the cerebral cortex.
2. **Wade H. Marshall,** *Johns Hopkins University*
Facilitation and recruitment during the subnormal phase in the geniculo-striate system of the cat.
3. **S. A. Talbot,** *Johns Hopkins University*
A lateral localization in cat's visual cortex.
4. **Charles Sheard and H. L. Bair** (*by invitation*), *Mayo Foundation and Clinic*
Rod and cone dark adaptation in total color blindness and in macular degeneration.
5. **Carney Landis and W. Edgar Vinacke** (*by invitation*), *New York Psychiatric Institute and Hosp.*
The discrimination of color and form at levels of illumination below conscious awareness.
6. **George Wald, Howard P. Krieger** (*by invitation*) **and Howard C. Goodman** (*by invitation*), *Harvard University, Cambridge*
Respiratory effects upon the visual threshold.
7. **T. Cuncliffe Barnes and T. L. Jahn** (*introduced by* R. Beutner), *Hahnemann Medical College and University of Iowa*
Electrical oscillations in a living membrane produced by vibrating tuning forks.
8. **Elmer A. Culler,** *University of Rochester*
A revised frequency-map of the guinea pig cochlea.
9. **Karl Lowy** (*introduced by* E. A. Culler), *University of Rochester*
Cancellation of the electrical cochlear response by simultaneous stimulation with air- and bone-conducted sound.
10. **J. C. R. Licklider and K. D. Kryter** (*introduced by* E. A. Culler), *University of Rochester*
Frequency-localization in the auditory cortex of the monkey.
11. **Robert Galambos** (*by invitation*) **and Hallowell Davis,** *Harvard Medical School*
The response of single auditory nerve fibers to acoustic stimulation.
12. **Edward M. Walzl** (*by invitation*) **and Clinton N. Woolsey,** *Johns Hopkins University*
Effects of cochlear lesions on click responses in the auditory cortex of the cat.
13. **Harlow W. Ades and Richard E. Felder** (*introduced by* E. A. Culler), *Emory University*
The primary cortical acoustic area of the monkey and the geniculo-temporal radiation.
14. **C. B. Weld and W. H. Feindel** (*by invitation*), *Dalhousie University*
The penetration of sugars in the aqueous humour.
15. **William M. Hart** (*introduced by* H. M. Hines), *State University of Iowa*
The distribution of lactic acid between the blood and aqueous humour and the rate of glycolysis of the crystalline lens.

PHYSIOLOGY B

Saturday 9:00 a.m.

Georgian Room

Blood Flow—Blood Pressure

1. **Alexander Kolin and David W. Fassett** (*introduced by* A. C. DeGraff), *New York University*
Studies on peripheral blood flow throughout the cardiac cycle; the electromagnetic method. (Pharmacol.)
2. **R. E. Shipley** (*by invitation*), **D. E. Gregg and J. T. Wearn,** *Western Reserve University*
Operative mechanism of some errors in the thermostromuhr method.
3. **W. H. Pritchard** (*by invitation*), **A. S. Weisberger** (*by invitation*), **E. F. Schroeder** (*by*

invitation), **R. E. Shipley** (*by invitation*) **and D. E. Gregg,** *Western Reserve University*
The acute effects of peripheral venous occlusion on blood flow in the dog's leg.

4. **D. E. Gregg, W. H. Pritchard** (*by invitation*) **and R. E. Shipley** (*by invitation*), *Western Reserve University*
Augmentation of right coronary inflow during pulmonary artery stenosis.

5. **H. E. Essex, J. F. Herrick, E. J. Baldes and F. C. Mann,** *Mayo Foundation*
Effect of exercise on the coronary blood flow, pulse rate and blood pressure of trained dogs with denervated and partially denervated hearts.

6. **Hampden Lawson,** *University of Louisville*
The effect of phasic contractions on the intestinal blood-flow.

7. **Eduardo Braun-Menendez** (*introduced by* Hallowell Davis), Univ. of Buenos Aires.
The secretion of renin by the normal kidney.

8. **A. C. Corcoran and Irvine H. Page,** *Indianapolis City Hospital*
The determinants of renal blood flow in hypertension.

9. **Eric Ogden, Ernest W. Page** (*by invitation*) **and G. J. Hildebrand** (*by invitation*), *University of California*
Relation of renin response and tachyphylaxis in acute renal hypertension.

10. **Arthur Grollman, T. R. Harrison** (*by invitation*) **and J. R. Williams, Jr.** (*by invitation*), *Wake Forest College*
Further studies on the preparation of kidney extracts effective in reducing the blood pressure in experimental hypertension.

11. **G. A. Wakerlin and** (*by invitation*) **C. A. Johnson, E. L. Smith, J. R. Weir and G. A. Olander,** *University of Illinois*
Prophylactic treatment of experimental renal hypertension with renin.

12. **John W. Bean,** *University of Buenos Aires*
On the specificity of renin and some related phenomena.

13. **Laurence W. Roth** (*by invitation*) **and Alrick B. Hertzman,** *St. Louis University*
The selective reactions of large and small finger arteries to local cold.

14. **John B. Dillon** (*by invitation*) **and Alrick B. Hertzman,** *St. Louis University*
The propagation of the pulse in the hand arteries.

15. **Grace M. Roth and Charles Sheard,** *Mayo Clinic and Foundation*
The effect of peripheral vasodilation on vasoconstriction: Determinations made on the basis of blood pressure of normal subjects.

16. **Joseph R. DiPalma** (*introduced by* G. B. Ray), *Long Island Medical College*
The effects of anoxemia, hypercapnia, acidosis, and alkalosis upon the threshold of reactive hyperemia in human skin resulting from local ischemia.

17. **David I. Abramson, Kamillo Flacks** (*by invitation*) **and Sidney M. Fierst** (*by invitation*), *May Institute*
Peripheral vascular responses in pregnancy.

PHYSIOLOGY C

Saturday 9:00 a.m.

PARLOR A

Muscle Physiology

1. **E. Newton Harvey and F. J. M. Sichel,** *Princeton University and University of Vermont*
A method of recording the dimensions of muscle fibre striations during contraction.

2. **Alexander Sandow** (*introduced by* R. Chambers), *Washington Square College*
Mechanical changes of muscle during the latent period of isometric contraction.

3. **Robert W. Ramsey and Sibyl F. Street** (*by invitation*), *University of Rochester*
Absence of fatigue of the contractile mechanism in single muscle fibers.

4. **George J. Hayes** (*by invitation*) **and Clinton N. Woolsey,** *Johns Hopkins University*
The unit of fibrillary activity and the site of origin of fibrillary contractions in denervated striated muscle.

5. **J. D. Thomson** (*by invitation*), **B. Lazere** (*by invitation*) **and H. M. Hines,** *State University of Iowa*
Quantitative studies on muscle and nerve regeneration.

6. **B. Lazere** (*by invitation*), **J. D. Thomson** (*by invitation*) **and H. M. Hines,** *State University of Iowa*
Studies on the glycogen metabolism of atrophic and regenerating muscle.

7. **Jacob Sacks and Charles H. Altshuler** (*by invitation*), *University of Michigan*
The distribution of radioactive phosphorus in striated and cardiac muscle. (Pharm.)

8. **W. O. Fenn and Lorraine F. Haege** (*by invitation*), *University of Rochester*
Exchange of potassium for ammonium in frog muscle.

9. **Lorin J. Mullins** (*introduced by* W. O. Fenn), *University of Rochester*
Selective accumulation of potassium by myosin.

10. **W. S. Wilde** (*introduced by* R. Ashman), *Louisiana State University*
The estimation of the sodium associated with extra-cellular acid groups of muscle.

11. **L. V. Heilbrunn and Pauline G. Hamilton** (*by invitation*), *University of Pennsylvania*
The chloride content of isolated muscle fibers.
12. **J. N. Stannard,** *Emory University*
Comparison of the effects of iodoacetate and iodoacetamide on the respiration of caffeinized frog muscle.
13. **Eliot Foltz** (*by invitation*), **A. C. Ivy and C. J. Barborka** (*by invitation*), *Northwestern University*
The use of double work periods in the study of fatigue and the influence of caffeine on recovery.
14. **George Maison and George Kotalik** (*by invitation*), *Wayne University*
Further investigation of the training pattern of human muscle.

PHYSIOLOGY D

Saturday 9:00 a.m.

BALLROOM

Symposium: The Mechanism of Secretion

A. C. Ivy, Chairman

1. **A. B. Dawson,** *Harvard University*
Some morphological aspects of the secretory process. (25 min.)
2. **Rudolf Höber,** *University of Pennsylvania*
Physicochemical aspects of the secretory process. (25 min.)
3. **M. B. Visscher,** *University of Minnesota*
The gastric and pancreatic secretion of dyestuffs in relation to their physicochemical characteristics. (25 min.)
4. **John S. Gray,** *Northwestern University*
The formation of acid and bicarbonate by secretory cells. (25 min.)
5. **J. Earl Thomas,** *Jefferson Medical College*
The effect of nervous and hormonal stimulation on zymogen secretion. (25 min.)

Discussion: **F. Hollander, H. W. Davenport, H. Necheles and A. J. Carlson** (5 min. ea.)
General Discussion.

PHYSIOLOGY E

Saturday 9:00 a.m.

SALLE MODERNE

Sex Hormones

1. **Lena A. Lewis and E. P. McCullagh** (*introduced by* D. R. McCullach), *Cleveland Clinic Foundation* (Biochem.)
Carbohydrate metabolism of methyl testosterone and testosterone propionate treated animals.
2. **Mark Graubard and Gregory Pincus,** *Clark University*
Estrogens and phenolases.
3. **Nicholas T. Werthessen** (*introduced by* Gregory Pincus), *Boston Dispensary and Pratt Diagnostic Hospital*
Estrogen excretion associated with dysmenorrhea.
4. **A. E. Rakoff** (*by invitation*), **K. Paschkis** (*by invitation*) and **A. Cantarow,** *Jefferson Medical College*
The demonstration of conjugated estrogens in human pregnancy serum, especially in the protein fraction.
5. **Warren O. Nelson,** *Wayne University*
Effects of estrogenic hormone in thyroidectomized rats.
6. **Albert Segaloff** (*by invitation*) **and Warren O. Nelson,** *Wayne University*
The role of the liver in the metabolism of a-estradiol.
7. **W. H. Pearlman** (*by invitation*) **and Gregory Pincus,** *Clark University*
The ketonic steroids of pregnancy urine.
8. **True W. Robinson and Henry C. Hill, Jr.** (*introduced by* C. G. Hartman), *University of Illinois*
Studies on induced ovulation in *Rana pipiens*.
9. **M. M. Hoffman** (*by invitation*) **and J. S. L. Browne,** *McGill University Clinic, Royal Victoria Hospital*
Further studies in the metabolism of progesterone.
10. **Eleanor Clarke** (*by invitation*) **and Hans Selye,** *McGill University*
Effect of various steroids on the vaginal mucosa.
11. **Helen Winter** (*by invitation*) **and Hans Selye,** *McGill University*
Factors influencing the diuretic effect of progesterone and desoxycorticosterone acetate.
12. **S. Albert** (*by invitation*) **and Hans Selye,** *McGill University*
The gonadotropic effect of various steroid compounds.
13. **C. P. Leblond** (*introduced by* H. Selye), *McGill University*
Detoxification of pregnanediol in the liver.
14. **G. Masson** (*by invitation*) **and Hans Selye,** *McGill University*
Factors regulating the lipid content of the rat endometrium.
15. **Clara M. Szego** (*by invitation*) **and Leo T. Samuels,** *University of Minnesota*
A colorimetric method for estrone and estradiol. (Pharmacol.)

PAPERS READ BY TITLE

PHYSIOLOGY

1. **Clifford A. Angerer** (*introduced by* Frank A. Hartman), *Ohio State University*
   Water content of sartorius of adrenalectomized frogs.
2. **Clifford A. Angerer and Helena Angerer** (*introduced by* Frank A. Hartman), *Ohio State University*
   The effect of dehydration on the viability of adrenal insufficient frogs.
3. **George Bachmann, John Haldi, Charles Ensor** (*by invitation*) **and Winfrey Wynn** (*by invitation*), *Emory University*
   Creatinuria following the ingestion of caffeine.
4. **John W. Bean,** *University of Michigan*
   The neuromyal junction as a peripheral contributor to the regulation of muscular activity.
5. **Richard J. Bing** (*by invitation*) **and Irvin M. Korr,** *New York University*
   The decarboxylation of dopa by extracts of kidney and other tissues of the rat.
6. **David F. Bohr** (*by invitation*) **and John W. Bean,** *University of Michigan*
   Hyperventilation as a retarding factor in oxygen poisoning.
7. **S. W. Britton and C. R. French** (*by invitation*), *University of Virginia*
   Cortico-adrenal extract influence on carbohydrate levels in thyroidectomized rats.
8. **S. W. Britton and R. F. Kline** (*by invitation*), *University of Virginia*
   On a condition simulating adrenal insufficiency on post-pituitary injected dogs.
9. **G. R. Bucher** (*by invitation*) **and H. Greengard,** *Northwestern University*
   Evidence against the alleged peptogogic effect of the hormone, secretin.
10. **M. W. Burrill** (*by invitation*) **and R. R. Greene,** *Northwestern University*
    The effectiveness of intramesenteric androgen pellets.
11. **Abraham Cantarow,** *Jefferson Medical College and Hospital*
    Quantitative studies of direct serum bilirubin.
12. **Emmett B. Carmichael,** *University of Alabama*
    Toxicity of pentothal sodium (sodium ethyl (1-methyl-butyl) thiobarbiturate) for guinea pigs.
13. **Emmett B. Carmichael, F. A. Kay** (*by invitation*) **and G. W. Phillips** (*by invitation*), *University of Alabama and Bryce Hospital*
    Development of tolerance in guinea pigs by repeated administration of large doses of paraldehyde.
14. **Helen C. Coombs,** *Columbia University*
    Calcium and potassium reversal effects of the perfused turtle heart.
15. **A. C. Corcoran, O. M. Helmer and Irvine H. Page,** *Indianapolis City Hospital*
    The renal pressor system as an index of species relationship.
16. **John Emerson Davis,** *University of Arkansas*
    The production of experimental polycythemia by the daily administration of epinephrine or posterior pituitary solution.
17. **Robert S. Dow and Robert Anderson** (*by invitation*), *University of Oregon*
    Cerebellar action potentials following proprioceptive and exteroceptive afferent stimulation in the rat.
18. **Alfred Gellhorn** (*by invitation*) **and Louis B. Flexner,** *Carnegie Institution of Washington*
    The transfer of water across the placenta of the guinea pig.
19. **E. Gellhorn and M. Kessler** (*by invitation*), *University of Illinois*
    The influence of hypoglycemia and electroshock on the electroencephalogram.
20. **Albert S. Gordon** (*by invitation*), **William Kleinberg** (*by invitation*) **and Harry A. Charipper,** *New York University*
    Effect of cobalt on anemia induced by x-ray in the guinea pig.
21. **Harold D. Green, Robert N. Lewis and Karl H. Radzow,** *Western Reserve University*
    The magnitude of the effective collateral circulation following sudden arterial occlusion.
22. **R. R. Greene and M. W. Burrill** (*by invitation*), *Northwestern University*
    48-hour response to androsterone in the immature male rat.
23. **S. C. Harris** (*by invitation*) **and J. S. Gray,** *Northwestern University*
    Some differences between urogastrone and enterogastrone.
24. **C. G. Holland** (*by invitation*), **R. F. Kline** (*by invitation*) **and S. W. Britton,** *University of Virginia*
    Sweat concentration after desoxycorticosterone injection.
25. **F. T. Jung, John M. Brady** (*by invitation*) **and Henry B. Upholt, Jr.** (*by invitation*), *Northwestern University*
    An attempt to correlate bleeding-time with capillary fragility in man.
26. **F. A. Kay** (*by invitation*), **Emmett B. Carmichael and G. W. Phillips** (*by invitation*), *Bryce Hospital and University of Alabama*
    Toxicity of paraldehyde for guinea pigs.
27. **Edward H. Lambert** (*by invitation*) **and George E. Wakerlin,** *University of Illinois*
    Failure to produce arterial hypertension by intracisternal injection of kaolin.

28. **Wesley E. Leonard** (*by invitation*) **and Chandler McC. Brooks,** *Johns Hopkins University*
Factors influencing micturition volume in the rat.

29. **Victor E. Levine,** *Creighton University* (Biochem.)
The blood pressure of the Eskimo.

30. **David I. Macht,** *Hynson, Westcott and Dunning, Inc.*
Influence of heparin on toxicity of ouabain and digitalis.

31. **David I. Macht,** *Hynson, Westcott and Dunning, Inc.*
Comparison of cobra venom and opiates on basal metabolism in man.

32. **David, I. Macht,** *Hynson, Westcott and Dunning, Inc.*
Absorption of epinephrine through the bone marrow.

33. **David I. Macht,** *Hynson, Westcott and Dunning, Inc.*
Effect of intramedullary injections of purgative and other oils.

34. **David I. Macht,** *Hynson, Westcott and Dunning, Inc.*
Effect of physical agents on stability of heparin.

35. **David I. Macht,** *Hynson, Westcott and Dunning, Inc.*
Duration of heparin effect in relation to method of administration.

36. **David I. Macht and Luceille Liston** (*by invitation*), *Hynson, Westcott and Dunning, Inc.*
Influence of tuberculin on behavior of rats and fishes.

37. **David I. Macht and Elizabeth C. Spencer** (*by invitation*), *Hynson, Westcott and Dunning, Inc.*
Pharmacodynamic synergism of cobra venom and thiamin.

38. **David I. Macht and Elizabeth C. Spencer** (*by invitation*), *Hynson, Westcott and Dunning, Inc.*
Effect of sulfanilamide derivatives on phytotoxic reactions of blood.

39. **David I. Macht, Elizabeth C. Spencer** (*by invitation*) **and Luceille Liston** (*by invitation*), *Hynson, Westcott and Dunning, Inc.*
Thromboplastic properties of some digitaloid drugs.

40. **Martin B. Macht** (*introduced by* Philip Bard), *Johns Hopkins University*
The relation of the level of decerebration to the type of response evoked by faradic stimulation of the anterior lobe of the cerebellum in the decerebrate cat.

41. **S. O. Mast and D. M. Pace** (*by invitation*), *Johns Hopkins University*
The effect of phosphorus on metabolism in *Chilomonas paramecium.*

42. **Jerome K. Merlis** (*introduced by* J. F. Fulton), *Yale University*
The influence of anesthesia and cortical lesions on vestibular nystagmus in the monkey.

43. **Jerome K. Merlis and Thomas A. Fraser** (*introduced by* J. F. Fulton), *Yale University*
The effect of ablation of the cerebellum on the cortically induced contractions of a pair of antagonistic muscles in the monkey.

44. **Fred A. Mettler,** *Columbia University*
Reticulocytosis as a sign of damage of the frontal lobe of the brain.

45. **Dorothy Nelson** (*introduced by* F. T. Jung), *Northwestern University*
The relation between increased weights of the heart and adrenals in experimental hyperthyroidism in the rat.

46. **O. S. Orth** (*by invitation*), **W. Vernon Lee** (*by invitation*) **and Walter J. Meek,** *University of Wisconsin*
The action of cyclopropane on the automatic tissues of the heart.

47. **G. W. Phillips** (*by invitation*), **Emmett B. Carmichael and F. A. Kay** (*by invitation*), *University of Alabama and Bryce Hospital*
Toxicity of paraldehyde for both young and old rats.

48. **E. Racker** (*introduced by* Herman Kabat), *Cardiff City Mental Hospital, England*
Observations on the metabolism of human fetal brain in vitro.

49. **A. D. Rankin** (*introduced by* H. H. Dukes), *Cornell University, Ithaca, N. Y.*
Studies on absorption from the rumen.

50. **R. Kohn Richards,** *Abbott Laboratories*
Toxicity and tolerance of para-amino benzoic acid.

51. **Herbert Silvette,** *University of Virginia*
Effect of posture on the duration of pentothal anesthesia.

52. **Howard Sloan and Rolf F. Klaus** (*introduced by* L. B. Nice), *Chicago University*
Concerning the toxic factor in traumatic shock.

53. **J. M. Werle** (*by invitation*), **R. S. Cosby** (*by invitation*) **and C. J. Wiggers,** *Western Reserve University*
Observations on hemorrhagic shock.

54. **C. Wigginton** (*by invitation*) **and K. B. Corbin,** *University of Tennessee*
Further observations on the lingual pseudomotor phenomenon.

55. **W. F. Windle and R. F. Becker,** *Northwestern University*
Some consequences of intrauterine asphyxiation in the guinea pig.

56. **W. B. Youmans, A. I. Karstens** (*by invitation*) **and K. W. Aumann** (*by invitation*), *University of Oregon*
Sensitization of intestinal smooth muscle to adrenalin by sympathetic denervation.

BIOCHEMISTRY A

Thursday, 2:00 p.m.

FOYER

Symposium on Vitamins and Enzyme Action

R. R. Williams, *Chairman*

1. **Carl F. Cori,** *Washington University*
The essential steps of carbohydrate metabolism.
2. **David E. Green,** *Columbia University*
The functions of thiamin and cocarboxylase.
3. **Otto E. Bessey,** *Harvard University*
Non-enzymic vitamins.
4. **E. E. Snell,** *University of Texas*
The biosynthesis of vitamins.

BIOCHEMISTRY B

Thursday, 2:00 p.m.

SWISS ROOM

Nitrogen Metabolism. I

(Amino Acids)

1. **E. H. Frieden, M. S. Dunn and C. D. Coryell,** *University of California, Los Angeles*
The reaction of formaldehyde with amino acids.
2. **Isidor Greenwald,** *New York University College of Medicine*
Some new compounds of zinc and glycine.
3. **W. C. Hess and M. X. Sullivan,** *Georgetown University*
Canine cystinuria. The cystine output on an arachin diet.
4. **Grace Medes and Norman F. Floyd,** *Lankenau Hospital*
Possible steps in the formation of taurine from cystine.
5. **Harold Tarver** (*by invitation*) **and Carl L. A. Schmidt,** *University of California Medical School*
Further studies employing radioactive sulfur.
6. **C. V. Smythe and D. Halliday** (*by invitation*), *University of Pittsburgh*
An enzymatic sulfur exchange between cysteine and radio-active sulfides.
7. **Anthony A. Albanese, Charlotte Kajdi and Dorothy M. Wangerin** (*introduced by* L. Emmett Holt, Jr.), *Johns Hopkins University*
Studies of experimental amino acid deficiency in man. II. Nitrogen and sulfur partition in the urine.
8. **Donald D. Van Slyke, Douglas A. MacFadyen** (*by invitation*) **and Paul Hamilton** (*by invitation*), *Rockefeller Institute for Medical Research, New York*
Application of the gasometric ninhydrin-CO_2 method to determination of amino acids in blood.
9. **Theodore F. Lavine,** *Lankenau Hospital*
A new method for the estimation of methionine.
10. **M. John Boyd and Milan A. Logan,** *University of Cincinnati Medical College*
Colorimetric determination of serine.
11. **F. W. Bernhart** (*by invitation*) **and D. Roy McCullagh,** *Cleveland Clinic Foundation*
The determination of tyrosine and related phenols in blood filtrates by means of the Millon reaction.
12. **M. X. Sullivan, W. C. Hess and H. W. Howard** (*by invitation*), *Georgetown University*
The estimation of cystine and cysteine together.

BIOCHEMISTRY C

Thursday, 2:00 p.m.

SHERATON ROOM

Carbohydrate Metabolism I

(Including phosphorus transfer)

1. **Zacharias Dische** (*introduced by* Harry Sobotka), *Mount Sinai Hospital, New York City*
Two forms of phosphorylation of glucose in human erythrocytes and their relation to the system cozymase—dihydrocozymase.
2. **Fritz Lipmann,** *Harvard Medical School*
Isolation of acetyl phosphate as precursor of acetic acid in pyruvate oxidation.
3. **Kurt G. Stern** (with the technical assistance of Jane Maurer), *Yale University*
On alcoholic fermentation as a heavy metal catalysis.
4. **Alfred Marshak,** *University of California, Berkeley*
Phosphorus metabolism of nuclei.
5. **Eunice V. Flock and Jesse L. Bollman,** *Institute of Experimental Medicine, Rochester, Minnesota*
Phosphate compounds labeled by radio-phosphorus in working and resting muscles of rats.

6. **W. W. Westerfeld** (*by invitation*), **Elmer Stotz, and Robert Berg** (*by invitation*), *Harvard Medical School and McLean Hospital, Waverley, Massachusetts*
   The role of pyruvate in the metabolism of ethyl alcohol.
7. **Ernest Bueding** (*by invitation*), **Joseph F. Fazekas** (*by invitation*) **and Harold E. Himwich,** *New York University Medical College and Union University, Albany, N. Y.*
   Action of insulin on pyruvate formation in depancreatized dogs.
8. **Karl Meyer, Henry S. Bloch** (*by invitation*), **and Eleanor Chaffee** (*by invitation*), *College of Physicians and Surgeons, Columbia University*
   The colorimetric determination of uronic acids.
9. **Alfred E. Koehler and Emanuel Windsor,** *Sansum Clinic and Santa Barbara Cottage Hospital*
   Ketone metabolism in the mouse.
10. **Marian E. Swendseid** (*by invitation*), **Allan Hemingway, Richard H. Barnes** (*by invitation*) **and A. O. Nier** (*by invitation*), *U. of Minnesota*
    The metabolism of acetic acid.
11. **Raymond Borchers** (*by invitation*) **and Clarence P. Berg,** *State University of Iowa*
    The effect of feeding tryptophane upon the urinary output of acetone bodies.
12. **Joseph S. Butts and Le Mar F. Remmert** (*by invitation*), *Oregon State College*
    Carbohydrate formation after feeding l(-)histidine.
13. **W. H. Peterson, Floyd C. McIntire** (*by invitation*) **and A. J. Riker** (*by invitation*), *University of Wisconsin*
    A polysaccharide produced by the crown-gall organism.

BIOCHEMISTRY A

Friday, 9:00 a.m.

FOYER

Symposium on the Metabolism of Protein

1. **H. J. Almquist,** *University of California*
   "The Amino Acid Requirements and Protein Metabolism of the Avian Organism."
   Discussion by **W. C. Rose,** *University of Illinois.*
2. **Philip Cohen,** *University of Wisconsin*
   "Transamination."
   Discussion by **Robert M. Herbst,** *New York University.*
3. **Clarence P. Berg,** *University of Iowa*
   "The Metabolism of the *d*-Amino Acids."
   Discussion by **J. R. Klein,** Duke University.
4. **Robert R. Sealock,** *University of Rochester*
   "The Relation of Vitamin C to the Metabolist of the Aromatic Amino Acids."
   Discussion by **H. B. Lewis,** *University of Michigan.*

BIOCHEMISTRY B

Friday, 9:00 a.m.

SWISS ROOM

Hormones and Steroids

(Including bile acids)

1. **Edward C. Kendall,** *Mayo Foundation*
   Quantitative studies of the activity of the hormones of the adrenal cortex.
2. **Eleanor H. Benning, M. M. Hoffman** (*by invitation*) **and J. S. L. Browne** (*by invitation*), *McGill University Clinic*
   Isolation of a 17-ketosteroid sulfate.
3. **J. Logan Irvin** (*introduced by* W. Mansfield Clark), *Johns Hopkins University*
   Partial synthesis of β-hyodesoxycholic acid and a procedure for the isolation of this bile acid from bile.
4. **L. H. Schmidt, H. B. Hughes** (*by invitation*) **and Willard M. Hoehn** (*by invitation*), *Christ Hospital, Cincinnati, O. and George A. Breon & Co., Kansas City, Missouri.*
   Bacterial oxidation of cholic acid.
5. **Robert W. Virtue,** *University of Denver*
   Production of cholic acid by the rat.
6. **Warren M. Sperry, Joseph W. Jailer** (*by invitation*), **and V. A. Stoyanoff** (*by invitation*), *New York State Psychiatric Institute and Hospital and Columbia University*
   A study of the cholesterol of the blood serum in monkeys.
7. **O. M. Helmer, K. G. Kohlstaedt** (*by invitation*), **G. F. Kempf and Irvine H. Page,** *Lilly Laboratory for Clinical Research, Indianapolis City Hospital*
   The assay of anti-pressor extracts of kidney by in vitro destruction of angiotonin.
8. **W. A. Robbie, A. H. Schein** (*by invitation*) **and R. B. Gibson,** *State University of Iowa*
   The clinical determination of urinary androgens.
9. **P. A. Katzman, Marcos Godfrid** (*by invitation*), **C. K. Cain** (*by invitation*) **and E. A. Doisy,** *St. Louis University*
   A new method for the preparation of chorionic gonadotropic hormone.

10. **Donald E. Bowman** (*introduced by* R. N. Hargar), *Indiana University*
Chemical differentiation between the chorionic gonadotropic hormone and serum proteins in aqueous solution.
11. **Fritz Bischoff and Georgena J. Clarke** (*by invitation*), *Santa Barbara Cottage Hospital*
Influence of nephrectomy on ovarian response to gonadotropins.
12. **Walter Mann, Charles Lebond, and Stafford Warren** (*introduced by* John R. Murlin), *University of Rochester*
Iodine metabolism in the thyroid gland.
13. **Leon S. Ciereszko** (*by invitation*) **and Abraham White,** *Yale University*
Isolation of a purified protein with marked thyrotropic activity from beef pituitary tissue.

BIOCHEMISTRY C

Friday, 9:00 a.m.

BALL ROOM

Fats and Phospholipids
Electrophoretic Studies on Proteins

1. **Walther F. Goebel, Mark H. Adams and Theodore Shedlovsky,** *Rockefeller Institute, New York*
The heterophile antigen of pneumococcus.
2. **Jordi Folch,** *The Rockefeller Institute, New York*
Brain cephalin, a mixture of phosphatides.
3. **Herbert E. Longenecker and Karl F. Mattil** (*by invitation*), *University of Pittsburgh*
Glyceride structure of rat body fats.
4. **P. L. MacLachlan** (*by invitation*), **Harold Carpenter Hodge, W. R. Bloor, Eileen A. Welch** (*by invitation*), **Frederick L. Truax** (*by invitation*) **and J. D. Taylor** (*by invitation*), *University of Rochester*
Lipids of the fasting mouse. II. The fat to water relation in the liver and the fractionation of the liver phospholipids.
5. **Camillo Artom and Marjorie Swanson** (*introduced by* W. R. Bloor), *Bowman Gray School of Medicine, Winston-Salem, N. C.*
The action of brominated fatty acids on liver fat.
6. **W. C. Stewart** (*by invitation*) **and R. G. Sinclair,** *Queen's University*
The metabolism of ricinoleic acid.
7. **Frieda Panimon Simon** (*by invitation*), **M. K. Horwitt and R. W. Gerard,** *Elgin State Hospital and University of Chicago*
Effect of hemins on the catalyzed oxidation of brain brei and phospholipids.
8. **Mona Spiegel-Adolf and George C. Henny** (*by invitation*), *Temple University School of Medicine*
X-ray diffraction studies in derivatives from lipids of tubercle bacilli. II
9. **Heinrich Waelsch and Marjorie Anchel** (*by invitation*), *College of Physicians and Surgeons, Columbia University*
The higher fatty aldehydes.
10. **Michael Somogyi and T. E. Weichselbaum,** *The Jewish Hospital of St. Louis*
Substrate competition in the utilization of ketone bodies.
11. **Zelma Baker Miller** (*by invitation*), **Henry M. Lemon** (*by invitation*) **and Benjamin F. Miller,** *University of Chicago*
Action of synthetic detergents on protein.
12. **John W. Mehl and Edwin L. Sexton** (*introduced by* Harry J. Deuel, Jr.), *University of Southern California*
Muscle proteins of the rat and of the octopus.
13. **Elvin A. Kabat, Harold Landow** (*by invitation*) **and Dan H. Moore** (*by invitation*), College of *Physicians and Surgeons, Columbia University*
An electrophoretic study of concentrated cerebrospinal fluid.

BIOCHEMISTRY A

Friday, 2:00 p.m.

FOYER

Symposium on Contributions of Physics to Biochemistry

1. **Francis O. Schmitt,** *Massachusetts Institute of Technology*
Molecular architecture of certain protein systems.
2. **Theodore Shedlovsky,** *Rockefeller Institute for Medical Research*
Electrophoresis in the study of proteins.
3. **D. W. Bronk and Frank Brink** (*to be presented by* Dr. Bronk), *University of Pennsylvania*
Energy requirements for the maintenance of structure and function in nerve.
Discussion to be opened by **Kurt G. Stern.**

BIOCHEMISTRY B

Friday, 2:00 p.m.

SWISS ROOM

Nitrogen Metabolism II
(Amino Acids and related subjects)

1. **Eliot F. Beach and** (*by invitation*) **Bertha Munks and Abner R. Robinson,** *Research Laboratory, Children's Fund of Michigan*
The amino acid composition of animal tissues.

2. **S. Gurin and D. Wright Wilson,** *University of Pennsylvania*
The intermediary metabolism of alanine containing C_{13}.
3. **William M. Cahill and Guilford G. Rudolph,** *Wayne University*
Some observations on the α-keto acid derived from methionine.
4. **Fred G. Brazda and James C. Rice** (*introduced by* H. H. Beard), *Louisiana State University*
Metabolism of N-derivatives of amino acids.
5. **Lynn De Forrest Abbott, Jr.** (*introduced by* J. C. Forbes), Medical College of Virginia
Availability of formylglycine, acetylglycine and propionylglycine for the synthesis of hippuric acid by the rabbit.
6. **Jakob A. Stekol,** *Fordham University and Vanderbilt University*
Further studies on the metabolism of benzyl chloride in the rat.
7. **Frank A. Csonka and Harry W. Titus,** *Bureaus of Agricultural Chemistry and Engineering and Animal Industry, U. S. Department of Agriculture*
Synthesis of amino acids by the chick embryo.
8. **L. Emmett Holt, Jr. and** (*by invitation*) **Anthony A. Albanese and Landrum B. Shettles, Charlotte Kajdi and Dorothy M. Wangerin,** *Johns Hopkins University*
Studies of experimental amino acid deficiency in man. I. Nitrogen balance.
9. **R. A. Phillips** (*by invitation*), **P. H. Futcher** (*by invitation*), **P. B. Hamilton** (*by invitation*), **R. M. Archibald** (*by invitation*), **A. E. Miller and D. D. Van Slyke,** *The Rockefeller Institute*
The source of the ammonia produced in the kidney in acidosis.
10. **Warren M. Cox, Jr., Arthur J. Mueller** (*by invitation*), **and K. S. Kemmerer** (*by invitation*), *Mead Johnson Laboratories*
Serum protein regeneration as effected by intravenously administered protein hydrolysates.
11. **Frederick W. Barnes and the late Rudolf Schoenheimer,** *Columbia University*
Relation of protein and purine metabolism in pigeons and rats.
12. **R. H. McCoy** (*by invitation*) **and M. O. Schultze,** *University of Pittsburgh*
Chemical studies on the bone marrow related to hematopoietic activity.
13. **Seymour S. Cohen** (*by invitation*) **and W. M. Stanley,** *The Rockefeller Institute, Princeton, N. J.*
The molecular size and shape of the nucleic acid of tobacco mosaic virus.

BIOCHEMISTRY C

Friday, 2:00 p.m.

SHERATON ROOM

Carbohydrate Metabolism II

(Including Tissue Oxidations)

1. **Charles O. Warren,** *Cornell University Medical College*
The Pasteur effect in bone marrow.
2. **William H. Summerson** (*introduced by* V. Du Vigneaud), *Cornell University Medical College*
The manometric measurement of acid production by cells and tissues in retentive media.
3. **B. Libet** (*by invitation*) **and K. A. C. Elliott,** *Pennsylvania Hospital*
A substance found in glycogen preparations and biotin concentrations which affects respiration.
4. **Frederick Bernheim,** *Duke University*
The oxidation of benzoate and related compounds by certain mycobacteria.
5. **Albert Dorfman, Sam Berkman and Stewart A. Koser** (*introduced by* F. C. Koch), *University of Chicago*
Pantothenic acid in the metabolism of *proteus morganii*.
6. **K. A. C. Elliott, D. B. McNair Scott** (*by invitation*) **and B. Libet** (*by invitation*), *Pennsylvania Hospital, Philadelphia, Pa.*
Combustion of glucose, lactate, pyruvate and non-carbohydrate material by brain tissue.
7. **Hans Gaffron and Jack Rubin,** *University of Chicago*
Photochemical production of hydrogen in green algae.
8. **Allan Hemingway, Marian Swendseid and A. O. Nier** (*introduced by* G. O. Burr), *University of Minnesota*
The use of heavy carbon as a tracer element.
9. **E. A. Evans, Jr., Louis Slotin** (*by invitation*) **and Birgit Vennesland** (*by invitation*), *University of Chicago*
Assimilation of radioactive carbon from $C^{11}O_2$ by various tissue preparations.
10. **Martin E. Hanke, John H. Bailey** (*by invitation*) **and Yale J. Katz** (*by invitation*), *University of Chicago*
Oxidation-reduction potential requirements and O_2 consumption of obligate anaerobes.
11. **Thomas B. Coolidge** (*introduced by* W. A. Perlzweig), *Duke University*
Poising of plasma.

12. **Ferrin B. Moreland** (*introduced by* H. A. Mattill), *State University of Iowa*
The effect of temperature on response to anoxia.

BIOCHEMISTRY A

Friday, 8:00 p.m.

SWISS ROOM

Round Table Conference

Joint Program with the Biometrics Section of the American Statistical Association
E. B. Wilson, *Chairman*

Statistical problems arising in research in experimental biology will be described by members of the Federation and discussed by members of the Biometrics Section in informal conference. In order to facilitate the discussion, members with problems for presentation are urged to send brief statements, preferably with illustrative data, to Secretary well in advance of the meeting. The first half or more of the Session will consider the application of the newer statistical designs and methods of analysis to problems selected from this list.

BIOCHEMISTRY A

Saturday, 9:00 a.m.

FOYER

Electrolytes and General Papers

1. **Oskar Baudisch and George Heggen** (*by invitation*), *Research Institute, Saratoga Spa*
Capture, concentration, quantitative determination and elimination of trace metals with p. tert. butyl-O-nitrosophenol (butyl-nitroso).
2. **David I. Hitchcock and Rozanne Peters** (*by invitation*), *Yale University*
The measurement and calculation of hydrogen ion concentrations in acetate and phosphate buffers.
3. **Elma Tufts Cohn** (*by invitation*) **and David M. Greenberg,** *University of California*
The biochemistry of mineral deficiency. III. Mineral balances.
4. **George H. Hitchings and Joseph T. Wearn** (*introduced by* Victor C. Myers), *Western Reserve University*
Chemical changes in the rabbit heart during hypertrophy.
5. **E. B. Man, E. F. Gildea and A. E. Smirnow,** *Yale University*
Serum iodine fractions.
6. **David M. Harkness** (*by invitation*) **Edward Muntwyler, Robert C. Mellors** (*by invitation*) **and Frederick R. Mautz** (*by invitation*), *Western Reserve University*
Electrolyte and water exchange between skeletal muscle, "available (thiocyanate) fluid", and plasma in the dog following the administration of desoxycorticosterone acetate.
7. **Alice Childs** (*by invitation*) **and Lillian Eichelberger,** *University of Chicago*
The distribution of water and electrolytes between blood, fluids and skeletal muscle in pregnant dogs.
8. **Marschelle H. Power, and** (*by invitation*) **Francis J. Robinson and Edwin J. Kepler,** *Mayo Foundation and Mayo Clinic*
Renal excretion of electrolytes by subjects with Addison's disease.
9. **Arnold E. Osterberg, and** (*by invitation*) **Della G. Drips, Edwin J. Kepler and Francis J. Robinson,** *Mayo Clinic*
Excretion of androgens by patients with Addison's disease.
10. **Kurt Salomon** (*by invitation*) **and George R. Cowgill,** *Yale University*
Porphyrinuria as a sign of lead poisoning: observations on dogs.
11. **Louis J. Polskin** (*by invitation*) **Bernard E. Simon** (*by invitation*), **Samuel Sklersky** (*by invitation*), **Albert E. Sobel and Alex Weinerman** (*by invitation*), (with the technical assistance of Henry Leonard, Raymond Liebling and George Kraus), *Jewish Hospital of Brooklyn*
Variations in the concentration of blood constituents following ligation of the hepatic ducts in dogs.
12. **M. F. Mason and R. E. Wheeler,** *Vanderbilt University*
Observations upon the toxicity of blue-green algae.

BIOCHEMISTRY B

Saturday, 9:00 a.m.

SWISS ROOM

Vitamins

1. **S. W. Clausen and A. B. McCoord** (*by invitation*), *University of Rochester*
The distribution of vitamin A, carotene and xanthophyll in the tissues of man and animals.
2. **W. C. Sherman** (*introduced by* W. D. Salmon), *Alabama Polytechnic Institute*
Influence of alpha-tocopherol and unsaturated fatty acids upon the utilization of vitamin A.

3. **Kenneth Morgareidge** (*introduced by* W. R. Bloor), *National Oil Products Company, Harrison, N. J.*
   Influence of solvent on the ultra violet absorption curve of vitamin A.
4. **C. A. Elvehjem and** (*by invitation*) **L. J. Tepley and A. E. Axelrod,** *University of Wisconsin*
   The effect of sulfapyridine on nicotinic acid metabolism.
5. **E. W. McHenry and Gertrude Gavin** (*by invitation*), *University of Toronto*
   Effects of inositol upon liver fat in the rat.
6. **Harold L. Mason and Ray D. Williams** (*by invitation*), *Mayo Foundation*
   Determination of thiamine in urine by the thiochrome method: estimation of the blank.
7. **Barnett Sure and Zenas Ford, Jr.,** (*by invitation*), *University of Arkansas*
   Thiamin and riboflavin metabolism.
8. **J. Raymond Klein and Henry Kamin** (*by invitation*), *Duke University*
   Effect of riboflavin on the metabolism of glucose and fructose by *lactobacillus helveticus*.
9. **Helen T. Parsons, Jean Collord** (*by invitation*), **Josephine Gardner** (*by invitation*), **F. M. Strong and W. H. Peterson,** *University of Wisconsin*
   Utilization by man of the vitamin B complex in fresh yeast.
10. **Gilbert M. Shull and Edward Nielsen** (*introduced by* W. H. Peterson), *University of Wisconsin*
    Intestinal synthesis of biotin and growth of rats on a low-biotin diet.
11. **W. D. Armstrong and C. P. Barnum** (*by invitation*), *University of Minnesota*
    A method of distinguishing endogenous and exogenous fecal phosphorus and the effect of vitamin D on phosphorus absorption.

BIOCHEMISTRY C

Saturday, 9:00 a.m.

SHERATON ROOM

Proteins and Enzymes

1. **Hans Neurath, Gerald R. Cooper** (*by invitation*), **John O. Erickson** (*by invitation*) **and D. Gordon Sharp** (*by invitation*), *Duke University*
   The heat denaturation of crystalline horse serum albumin.
2. **Erwin Brand and Beatrice Kassell,** *Columbia University*
   Minimum molecular weight (M_{min}) of β-lactoglobulin.
3. **Thomas L. McMeekin,** *Bureau of Agricultural Chemistry and Engineering, U. S. Department of Agriculture*
   Precipitation of proteins by organic sulfates and sulfonates.
4. **Gerrit Toennies,** *Lankenau Hospital Research Institute*
   The conversion of casein into a methionine and tryptophane deficient protein by an oxidative treatment.
5. **Jacinto Steinhardt,** *National Bureau of Standards*
   Catalysis by anions of the hydrolysis of amide and peptide bonds in proteins.
6. **H. S. Olcott and L. A. Sapirstein** (*by invitation*), *Bureau of Agricultural Chemistry and Engineering, U. S. Department of Agriculture*
   Proteinase of wheat gluten.
7. **M. K. Horwitt,** *Elgin State Hospital, Elgin, Illinois*
   A study of the first stages of casein hydrolysis by chymotrypsin and trypsin.
8. **Joseph S. Fruton and Max Bergmann,** *The Rockefeller Institute, New York*
   The specificity of chymotrypsin.
9. **William F. Ross and Ann H. Tracy,** *Radcliffe College*
   A comparison of the reaction of carbon suboxide on proteins with that of ketene and the digestion of malonyl albumins by chymotrypsin.
10. **Donald D. Van Slyke and** (*by invitation*) **Reginald M. Archibald** (to be given by Dr. Archibald), *The Rockefeller Institute, New York*
    Purification, kinetics and activity measurement of liver arginase.
11. **D. A. Scott and A. M. Fisher,** *University of Toronto*
    Carbonic anhydrase.
12. **Jesse P. Greenstein,** *National Institute of Health*
    Ribonuclease and thymonucleodepolymerase.
13. **Burnham S. Walker,** *Massachusetts Memorial Hospitals, and Boston University School of Medicine*
    Direct measurement of catalatic activity with the dropping mercury electrode.

PAPERS READ BY TITLE

BIOCHEMISTRY

1. **Carl E. Anderson** (*by invitation*) **and James C. Andrews,** *University of North Carolina*
   The enzymatic conversion of quinine in vitro by tissues of the rat.

2. **James C. Andrews and Carl E. Anderson** (*by invitation*), *University of Carolina*
   The enzymatic conversion of quinine in vivo in the rat.
3. **James C. Andrews, W. E. Cornatzer** (*by invitation*) **and A. B. Sample** (*by invitation*), *University of North Carolina*
   The extraction of proteins from aqueous solution by means of emulsification with chloroform.
4. **A. K. Balls, Bernard Axelrod** (*by invitation*) **and Marian W. Kies** (*by invitation*), *U. S. Bureau of Agricultural Chemistry and Engineering*
   Inhibition of carotene oxidase.
5. **Clifford D. Bauer** (*by invitation*) **and Clarence P. Berg,** *University of Iowa*
   The utilization of amino acids for growth in the mouse.
6. **Vincent P. Calkins** (*by invitation*) **and H. A. Mattill,** *University of Iowa*
   The antioxygenic action of ascorbic acid with quinone.
7. **W. Wesley Campbell** (*by invitation*) **and Wilbur R. Tweedy,** *Loyola University*
   The effect of parathyroid extract upon the distribution and excretion of labeled phosphorus.
8. **Thomas B. Coolidge** (*introduced by* W. A. Perlzweig), *Duke University*
   The Van den Bergh reaction.
9. **James Flexner** (*by invitation*), **Maurice Bruger and Samuel Member** (*by invitation*), *Columbia University*
   The excretion of iodine by the salivary and gastric glands.
10. **Alfred H. Free** (*by invitation*), **Dean F. Davies** (*by invitation*), **Gordon E. Gustafson** (*by invitation*) **and Victor C. Myers,** *Western Reserve University*
    Studies on continued alkali ingestion in man.
11. **Theodore E. Friedemann and Thaddeus C. Kmieciak** (*by invitation*), *Northwestern University*
    The determination of thiamin in blood.
12. **Theodore E. Friedemann and Thaddeus C. Kmieciak** (*by invitation*), *Northwestern University*
    The excretion of thiamin.
13. **Theodore E. Friedemann, S. C. Werch** (*by invitation*) **and Gladys E. Haugen** (*by invitation*), *Northwestern University*
    The metabolism of pyruvic acid.
14. **Max M. Friedman** (*introduced by* Bernard B. Brodie), *Fordham Hospital*
    Simplified bromide determination for extracellular fluid measurement.
15. **L. E. Gilson** (*by invitation*), **A. A. Tytell** (*by invitation*), **M. A. Logan, and Patricia Perkins** (*by invitation*), *University of Cincinnati*
    Oxalic acid hydrolysate of casein for bacteriological purposes.
16. **Isidor Greenwald,** *New York University*
    The effect of pH and of the amount of solid phase upon the solubility of calcium phosphate.
17. **O. M. Helmer, K. G. Kohlstaedt** (*by invitation*) **and Irvine H. Page,** *Indianapolis City Hospital*
    Destruction of angiotonin by extracts of various tissues.
18. **William S. Hoffman,** *Chicago Medical College*
    A dilution technique for the photoelectric analysis of a mixture of two colors.
19. **Franklin Hollander, Edward E. Jemerin** (*by invitation*) **and Vernon A. Weinstein** (*by invitation*), *Mt. Sinai Hospital*
    An insulin test for differentiating vagal from non-vagal stomach pouches
20. **H. M. Kalckar,** *Washington University*
    Myokinase and adenylpyrophosphate.
21. **Granvil C. Kyker** (*introduced by* James C. Andrews), *University of North Carolina*
    The response of the cinchonas and other bases to the photelmetric estimation of quinine.
22. **Ferrin B. Moreland** (*introduced by* H. A. Mattill), *University of Iowa*
    The preparation of colorimetric standards.
23. **Frederick S. Philips** (*introduced by* Kurt G. Stern), *Yale University*
    Photochemical spectrum of the respiratory ferment in a tissue of the chick embryo.
24. **Marschelle H. Power,** *Mayo Foundation*
    Colorimetric determination of magnesium.
25. **Roger M. Reinecke** (*introduced by* Edward C. Kendall), *Mayo Foundation*
    A bioassay for the effect of the hormones of the adrenal cortex on carbohydrate metabolism.
26. **Peter W. Salit, Kenneth C. Swan, and William D. Paul** (*introduced by* R. B. Gibson), *University of Iowa*
    Changes in mineral composition of rat lenses with galactose cataract.
27. **Shiro Tashiro,** *University of Cincinnati*
    The effect of bile salts on chromodacryorrhetic action of acetylcholine.

PHARMACOLOGY A

Wednesday, 2:00 p.m.

BALL ROOM

Analgesics, Opiates

1. **David R. Climenko,** *Winthrop Chemical Co., Inc.*
   1-Methyl 4-phenylpiperidine-4-carbonic acid

ethyl ester (D-140, demerol): A pharmacologically active substance with atropine-like and morphine-like properties. (Physiol.)

2. **Robert C. Batterman,** *New York University College of Medicine*
   The effectiveness in man of demerol, a new analgesic drug.
3. **H. L. Andrews** (*introduced by* C. K. Himmelsbach), *U. S. P. H. S. Hospital, Lexington*
   The development of tolerance to demerol.
4. **C. K. Himmelsbach,** *U. S. P. H. S. Hospital, Lexington*
   The addiction liability of demerol (1-methyl-4-phenyl-piperidine-4-carbonic acid ethyl ester).
5. **J. K. Theisen and F. E. Shideman** (*introduced by* M. H. Seevers), *University of Wisconsin*
   The coenzyme I content of liver and skeletal muscle of chronically morphinized rats.
6. **F. E. Shideman** (*by invitation*) **and M. H. Seevers,** *University of Wisconsin*
   The effect of chronic morphine poisoning on the oxygen uptake of dog skeletal muscle.
7. **Carroll A. Handley** (*by invitation*) **and Benedict E. Abreu,** *University of California Medical School, University of South Dakota School of Medicine and University of Georgia School of Medicine*
   Effects of benzedrine on oxygen consumption after morphine in men and rats.
8. **Julian B. Herrmann,** *Yale University School of Medicine*
   The pyretic action on rats of small doses of morphine.
9. **Donald Slaughter and** (*by invitation*) **J. W. Gales,** *Baylor University College of Medicine*
   The pain threshold response to morphine in humans as modified by prostigmin methylsulfate.
10. **Edwin G. Williams** (*introduced by* C. K. Himmelsbach), *U. S. P. H. S. Hospital, Lexington*
    Serum cholinesterase activity in relation to morphine addiction in man.
11. **Fred W. Oberst,** *U. S. P. H. S. Hospital, Lexington*
    Urinary excretion of heroin (diacetylmorphine). (Biochem.)

PHARMACOLOGY B

Wednesday, 2:00 p..m

CASINO

Autonomic and Related Drugs

1. **L. D. Seager,** *University of Tennessee*
   The response of the rat uterus to sympathomimetic drugs.
2. **Georg Barkan,** *Evans Memorial, Massachusetts Memorial Hospitals and Boston University School of Medicine*
   Ejaculation induced by a uterine drug.
3. **William E. Evans, Jr.,** *University of Maryland School of Medicine*
   A uterine principle from viburnum prunifollium.
4. **Edwin J. Fellows and Raymond W. Cunningham,** *Temple University School of Medicine*
   The effect of a number of new phenyl-piperidine derivatives on smooth muscle.
5. **Raymond W. Cunningham and Edwin J. Fellows,** *Temple University School of Medicine*
   The antispasmodic action of a series of new derivatives of isoquinoline.
6. **A. J. Lehman and H. F. Chase** (*by invitation*), *Wayne University Collège of Medicine*
   The anticonvulsant action of natural and synthetic curare-like agents.
7. **Ralph G. Smith,** *University of Michigan Medical School*
   The application of the photoelectric colorimeter for the determination of ergot alkaloids.
8. **Carl Pfeiffer and George L. Maison** (*by invitation*), *Wayne University College of Medicine*
   Ergotamine tartrate on work capacity of human muscle.
9. **Sydney Ellis** (*introduced by* Otto Krayer), *Harvard Medical School*
   The inhibition of cholinesterase by breakdown products of physostigmine.
10. **Clara Torda** (*introduced by* Harry Gold), *Cornell University Medical College*
    The actions of ether and chloroform in relation to the choline esterase mechanism.
11. **Gerhard Katz,** *Tulane University of Louisiana*
    Histamine release in the allergic skin reaction.
12. **Albert J. Plummer** (*introduced by* W. L. Mendenhall), *Boston University School of Medicine*
    The antagonism between nicotine and physostigmine.

PHARMACOLOGY

Wednesday, 8:00 p.m.

BALL ROOM

SYMPOSIUM

Morphine Problems

Nathan B. Eddy, *Chairman*

H. G. Wolff, *New York Hospital.* Pain and its relief by morphine and related substances.

C. K. Himmelsbach, *U. S. P. H. S. Hospital.* The present status of morphine addiction studies.

E. G. Gross, *State University of Iowa.* The fate in the body of morphine and related substances.

M. H. Seevers, *University of Wisconsin.* The effect of morphine and related substances on tissue metabolism.

PHARMACOLOGY A

Thursday, 2:00 p.m.

BALL ROOM

Toxicity, Tolerance

1. **Raymond M. Selle** (*introduced by* Clinton H. Thienes), *Occidental College*
   Effects of subcutaneous injections of sodium tungstate on the rat.
2. **Ruth Cortell and R. Kohn Richards** (*introduced by* E. M. K. Geiling), *Abbott Laboratories*
   Studies on the tolerance formation to gold salts in rats.
3. **Ralph B. Hogan and Harry Eagle** (*introduced by* E. K. Marshall, Jr.), *U. S. Public Health Service, and the Johns Hopkins Medical School*
   A suggested pharmacological basis for the widely varying toxicity of arsenicals.
4. **J. H. Draize and E. P. Laug** (*by invitation*), *Food and Drug Administration, Federal Security Agency*
   The percutaneous absorption of ammonium hydrogen sulfide and hydrogen sulfide.
5. **Theodore Koppanyi and C. R. Linegar,** *Georgetown University School of Medicine*
   Contributions to the pharmacology of sulfides.
6. **Byron B. Clark and Edward J. Van Loon** (*by invitation*), *Albany Medical College*
   The acute effects of hypoxia resulting from methemoglobin produced by aniline.
7. **D. D. Donahue, J. L. Svirbely** (*by invitation*), **P. J. Valaer** (*by invitation*) **and W. F. von Oettingen,** *Division of Industrial Hygiene, National Institute of Health*
   The effect of ingestion of glycine and ascorbic acid on the toluene level in blood, the excretion of hippuric acid, and the blood picture of dogs exposed to various concentrations of toluene.
8. **W. F. von Oettingen, D. D. Donahue, and P. J. Valaer and J. W. Miller** (*by invitation*), *Division of Industrial Hygiene, National Institute of Health*
   Studies on the treatment of experimental carbon monoxide poisoning.
9. **Wilhelm Deichmann and Karl V. Kitzmiller** (*by invitation*), *University of Cincinnati Medical College*
   Toxic effects upon rabbits and cats of pentachlorophenol.
10. **James L. Morrison** (*introduced by* George A. Emerson), *University of California Medical School, and the West Virginia University School of Medicine*
    Biochemorphology of halogenated fatty acids.
11. **Paul N. Harris and Robert C. Anderson** (*by vitation*) **and K. K. Chen,** *Lilly Research Laboratories*
    The action of monocrotaline, isatidine, pterophine, sceleratine, and the alkaloid of senecio carthamoides. (Physiol.)
12. **R. Kohn Richards,** *Abbott Laboratories*
    Potentiation of epinephrine toxicity by sodium bisulfite. (Physiol.)
13. **H. Wastl,** *Hahnemann Medical College*
    Influence of various salts on the toxicity of pontocaine hydrochloride. (Physiol.)

PHARMACOLOGY B

Thursday, 2:00 p.m.

CASINO

General Anesthetics, Hypnotics

1. **Nellie M. Cone** (*by invitation*) **and C. Jelleff Carr,** *University of Maryland School of Medicine*
   A study of the relationship between anesthetic potency and physical properties.
2. **John C. Krantz, Jr., and Sylvan E. Forman** (*by invitation*), *University of Maryland School of Medicine*
   The pharmacology and anesthetic properties of cyclopropyl vinyl ether.
3. **Stevens J. Martin** (*by invitation*) **and Amedeo S. Marrazzi,** *New York University College of Medicine*
   The action of cyclopropane and chloroform on the central origins of the sympathetic system.
4. **Paul Dumke and Robert Dripps** (*introduced by* Carl F. Schmidt), *University of Pennsylvania*
   Influence of anesthetics on balance between chemoreceptor and central control of respiration.
5. **J. H. Barbour** (*by invitation*) **and M. H. Seevers,** *University of Wisconsin*
   The effects of various concentrations of carbon dioxide on the toxicity of ethyl (1-methyl butyl) barbituric acid.
6. **C. H. Hine, J. H. Holman** (*by invitation*) **and F. L. Kozelka,** *University of Wisconsin*
   Metabolism of some derivatives of dilantin.
7. **F. L. Kozelka, and C. H. Hine and M. F. Greiber** (*by invitation*), *University of Wisconsin*

Anticonvulsant properties of the metabolic products and some derivatives of dilantin.

8. **Melvin W. Green** (*by invitation*) **and Theodore Koppanyi**, *Georgetown University School of Medicine*
   Tolerance and cross tolerance to barbiturates.
9. **Emmett B. Carmichael,** *University of Alabama School of Medicine*
   Development of cross tolerance to both pentobarbital sodium and delvinal sodium by a single large injection of pentothal sodium in guinea pigs. (Physiol.)
10. **S. A. Peoples,** *University of Alabama School of Medicine*
    The effect of dinitrophenol and thyroxin on the duration of sodium pentabarbital anesthesia in rats.
11. **Hans Selye,** *McGill University*
    Further studies concerning the anesthetic action of steroid compounds. (Physiol.)
12. **Edmund Jacobson,** *Laboratory for Clinical Physiology, Chicago*
    Direct measurements on the influence of caffeine, bromides and sodium amytal in man. (Physiol.)

PHARMACOLOGY A

Friday, 9:00 a.m.

BALL ROOM

Cardiac Glycosides

1. **James M. Dille and George B. Whatmore** (*by invitation*), *University of Washington, Seattle*
   The gastro-intestinal absorption of lanatoside C.
2. **Melvin Dresbach,** *University of Pennsylvania*
   Emetic responses of glycosides as evidence of their cumulation and synergistic action in the central nervous system. (Physiol.)
3. **Janet Travell, Harry Gold, Walter Modell** (*by invitation*) **and Oscar Auerbach** (*by invitation*), *Cornell University Medical College, and Sea View Hospital*
   A study of cardiac hemorrhages caused by the digitalis glycosides.
4. **Enrique Moisset de Espanés** (*introduced by* Otto Krayer), *Harvard Medical School*
   The action of unsaturated lactones on the isolated frog heart.
5. **K. K. Chen and Robert C. Elderfield** (*by invitation*), *Lilly Laboratories and Columbia University*
   Unsaturated lactones related to cardiac glycosides. (Physiol.)
6. **Harald G. O. Holck, and E. L. Smith, R. H. Shuler, R. Van Norman, D. R. Mathieson, and J. Dahlstet** (*by invitation*), *University of Nebraska*
   Statistical studies upon the effects of anesthetics in the intravenous cat method of standardizing digitalis.
7. **Rafael Mendez** (*introduced by* Otto Krayer), *Harvard Medical School*
   The use of the spinal cat for the estimation of the potency of cardiac glycosides.
8. **Robert A. Lehman and George H. Paff** (*introduced by* Arthur C. DeGraff), *New York University College of Medicine*
   A practical technique and design for the assay of digitalis on the embryonic chick heart.
9. **Harry Gold, and Harold Otto and Nathaniel T. Kwit** (*by invitation*), *Cornell University Medical College and the Cardiac Clinics of the Hospital for Joint Diseases and Beth Israel Hospital*
   A method for the bio-assay of digitalis in humans.
10. **Stephen Krop** (*introduced by* McKeen Cattell), *Cornell University Medical College*
    The action of the xanthines on the force of contraction of isolated mammalian heart muscle.
11. **McKeen Cattell,** *Cornell University Medical College*
    The influence of ouabain on the "efficiency" and force of contraction of striated muscle.
12. **Gordon K. Moe** (*introduced by* Otto Krayer), *Harvard Medical School*
    The action of veratridine upon the heart-lung preparation.
13. **Otto Krayer,** *Harvard Medical School*
    The effect of veratrum alkaloids on circulatory reflexes.
14. **Ludwig W. Eichna and H. Taube** (*introduced by* J. Ernest Nadler), *New York College of Medicine*
    Serial determinations of cardiac output (ballistocardiograms) and electrocardiogram in normal man after the administration of digitalis glycosides.

PHARMACOLOGY B

Friday, 9:00 a.m.

CASINO

Chemotherapy, Amines, Circulatory Drugs

1. **Clyde Brooks and Harvey B. Searcy** (*by invitation*), *Louisiana State University*
   Experimental and clinical studies on the effect of methyl-isopropyl-phenol on tuberculosis.
2. **Harry Beckman,** *Marquette University School of Medicine*
   The effect of multiple mosquito bites on the course of plasmodium cathemerium infection in canaries.

3. **George A. Pastorius** (*by invitation*) **and Paul L. McLain,** *University of Pittsburgh School of Medicine*
   On the mode of action of toxic doses of hydroxyethylapocupreine.
4. **R. S. Shelton, M. R. Warren, T. J. Becker, and M. G. Van Campen** (*introduced by* M. H. Seevers), *Research Laboratories, The Wm. S. Merrell Company*
   Central stimulation studies on a number of amines.
5. **T. J. Becker, M. R. Warren, D. G. Marsh, C. R. Thompson and R. S. Shelton** (*introduced by* M. H. Seevers), *Research Laboratories, The Wm. S. Merrell Company*
   Preliminary studies on the pharmacology of dl-B-phenyl-n-propylmethylamine.
6. **C. H. Thienes, George J. Majovski and Carl Schubert** (*by invitation*), *University of Southern California*
   Cutaneous vascular permeability and fragility in rabbits as influenced by lemon citrin.
7. **R. A. Escobar and H. F. Wilkins** (*by invitation*) **and C. H. Thienes,** *University of Southern California*
   Effects on coronary circulation of isomers of neosynephrine and of amphetamine.
8. **R. A. Woodbury and W. F. Hamilton,** *University of Georgia School of Medicine*
   Cardiovascular effects of pituitary preparations which contain the oxytocic principle.
9. **Margaret E. M. Sawyer and G. H. Ettinger** (*by invitation*), *Queen's University*
   The action of posterior pituitary extract on the dog heart. (Physiol.)
10. **B. E. Abreu and R. A. Woodbury,** *University of Georgia School of Medicine*
    An analysis of the blood pressure and respiratory changes produced by strychnine convulsions.
11. **W. Lloyd Adams and C. Stuart Welch** (*by invitation*), **and Byron B. Clark,** *Albany Medical College*
    Use of the cope pouch dog for the study of the effect of gastric antacids on gastric secretion.

PHARMACOLOGY

Friday, 2:00 p.m.

SYMPOSIUM

Deficiency Diseases

BALL ROOM

Tom D. Spies, *Chairman*

C. G. King, *University of Pittsburgh.* Some specific physiological disturbances induced by marginal vitamin deficiencies (C and B_1).

F. C. Bing, *American Medical Association.* Food as a source of vitamins.

C. A. Elvehjem, *University of Wisconsin.* The biological action of the vitamins.

Hans Molitor, *Merck Institute for Therapeutic Research.* Vitamins as pharmacological agents.

Norman Jolliffe, *New York University College of Medicine.* Vitamins in the practice of medicine.

W. H. Sebrell, *National Institute of Health.* Vitamins in public health.

PHARMACOLOGY A

Saturday, 9:00 a.m.

BALL ROOM

Hormones, Excretion, Convulsants

1. **Gilberto Rivera** (*introduced by* Clyde Brooks), *Tropical School of Medicine, University of Puerto Rico, under auspices of Columbia University, San Juan, Puerto Rico*
   Studies on planta cundeamor.
2. **M. K. McPhail and H. C. Read** (*by invitation*), *Dalhousie University*
   Adrenal gland: sensitivity of the X-zone to various hormonal substances.
3. **Kathryn Knowlton** (*by invitation*), **Irene Sandiford and Allan T. Kenyon,** *University of Chicago*
   The comparative metabolic effects of testosterone propionate and estradiol benzoate in man.
4. **Norman A. David, Nilkanth Phatak and Rosa Kubin** (*by invitation*), *University of Oregon Medical School*
   Placental transmission of blood iodine.
5. **P. S. Larson and H. B. Haag** (*by invitation*), *Medical College of Virginia*
   Studies on the fate of nicotine in the dog. (Physiol.)
6. **H. B. Haag and P. S. Larson,** *Medical College of Virginia*
   Urinary excretion of nicotine by smokers.
7. **John F. Johnson** (*by invitation*) **and Claude V. Winder,** *University of Michigan*
   Immediate effects of tobacco smoke on the dog's respiration and blood pressure. (Physiol.)
8. **Werner L. Lipschitz and Zareh Hadidian** (*introduced by* S. Loewe), *Lederle Laboratories*
   Bioassay of diuretics.
9. **P. A. Nuhfer, C. H. Mellish and J. A. Buchter** (*introduced by* Harry Beckman), *The Lakeside Laboratories, Inc.*
   The toxicity, absorption and diuretic effect of a mercurated allylsuccinylurea with theophylline.

10. **R. Beutner and B. Calesnick** (*by invitation*), *Hahnemann Medical College*
The convulsive action of the commonly used local anesthetics.
11. **Gabriel Fournier** (*by invitation*) **and Hans Selye,** *McGill University*
Concerning the site of pentamethylenetetrazol (metrazol) detoxification. (Physiol.)

PHARMACOLOGY B

Saturday, 9:00 a.m.

CASINO

Sulfonamides

1. **George W. Raiziss, M. Severac and J. C. Moetsch,** *University of Pennsylvania, and the Abbott Laboratories, Philadelphia Division*
Sulfapyrazine—a new chemotherapeutic agent. (Biochem.)
2. **A. D. Welch** (*introduced by* A. D. Hirschfelder), *Medical Research Division, Sharp and Dohme, Inc.*
Succinyl-sulfathiazole ('sulfasuxidine') as an inhibitor of bacterial syntheses in nutrition experiments.
3. **Paul A. Mattis and A. D. Welch** (*introduced by* A. D. Hirschfelder), *Medical Research Division, Sharp and Dohme, Inc.*
Toxicological studies on succinyl-sulfathiazole ('sulfasuxidine').
4. **Versa V. Cole and Ben King Harned,** *Woman's College of Pennsylvania*
The effects of a diet deficient in vitamin B on the toxicity of sulfapyridine.
5. **Frederick F. Yonkman and** (*by invitation*) **Frank Bruce, Marshall Purdy, Garnett Ice and John Jacobs,** *Wayne University College of Medicine*
The irritating properties of a propylene glycol solution of sulfanilamide.
6. **A. M. Ambrose and R. Arnold Griswold** (*by invitation*), *University of Louisville School of Medicine*
Absorption of sulfadiazine after intraperitoneal administration in man.
7. **Eldon M. Boyd,** *Queen's University*
The effect of sulphonamides upon peptone fever.
8. **Arthur P. Richardson,** *University of Tennessee Medical School*
The nature of Heinz bodies and their relation to sulfanilamide chanosis and anemia in mice.
9. **C. Schlayer** (*by invitation*), **W. Kempner and P. Summers** (*by invitation*), *Duke University*
Inhibition of the sulfonamide effect on pneumococcus by P-aminobenzoic acid in the absence of oxygen. (Physiol.)
10. **Walter Kempner,** *Duke University*
Sulfonamide action on pneumococcus and staphylococcus at various oxygen concentrations and in the absence of oxygen, determined by measuring manometrically the bacterial metabolism. (Physiol.)
11. **Henry I. Kohn and Jerome S. Harris** (*by invitation*), *Duke University*
Anti-sulfanilamide activity of glycine, serine and allothreonine in E. coli. (Physiol.)
12. **Julia B. MacKenzie and C. G. MacKenzie** (*introduced by* E. V. McCollum), *School of Hygiene and Public Health, Johns Hopkins University*
The effect of "sulfa" drugs on the thyroid gland in rats and mice. (Biochem.)
13. **Esther M. Greisheimer, Roberta Hafkesbring and Grace E. Wertenberger** (*by invitation*), *Woman's Medical College of Pennsylvania*
The effect of sodium sulfadiazine, sulfadiazine and sulfaguanidine on blood sugar and liver glycogen. (Physiol.)
14. **Frederick Steigmann, Hans Popper and Hattie Dyniewicz** (*introduced by* Hugh McGuigan), *University of Illinois College of Medicine and Cook County Hospital, and Cook County Graduate School of Medicine*
Influence of phenolphthalein and sulfanilamide on the vitamin A distribution in the liver.

PAPERS READ BY TITLE

PHARMACOLOGY

1. **A. M. Ambrose,** *University of Louisville School of Medicine*
Absorption of sulfadiazine after intraperitoneal administration in dogs.
2. **Hamilton H. Anderson and Peter P. T. Sah,** *Peiping Union Medical College*
Anthelmintic action of certain halogenated hexyl and hexoyl resorcinols.
3. **R. Beutner and B. Calesnick,** *Hahnemann Medical College, Philadelphia*
The local action of mercurial diuretics.
4. **R. Beutner and A. Cohen,** *Hahnemann Medical College, Philadelphia*
The pharmacological properties of orthonitrophenol.
5. **Josephine Campbell,** *University of California Medical School*
Toxicity and antiseptic action of cresylic acids.

6. **Y. C. Chin and Hamilton H. Anderson,** *Peiping Union Medical College*
   Larvicidal action of chloro-hexyl-meta-cresol.
7. **T. K. Ch'uan,** *Peiping Union Medical College*
   The activity of ya-tan-tzu as an amebacide in vitro.
8. **Helen C. Coombs and F. H. Pike,** *Brooklyn College and Columbia University*
   Some effects of depressants of the central nervous system on the nembutalized cat.
9. **Carl A. Dragstedt, J. A. Wells and M. Rocha E. Silva,** *Northwestern University Medical School, Chicago*
   Heparin as an antidote to trypsin in the rat.
10. **J. K. Finnegan, E. L. McCawley and L. A. Strait,** *University of California Medical School*
    Comparative toxicity of kerosenes.
11. **Paul G. Fuerstner,** *University of California Medical School*
    Reaction of fallopian tubes and ovaries of rabbits to repeated administration of pitressin NNR.
12. —— **Gurchot and Michael Joseph,** *University of California Medical School*
    The papain activating power of blood in health and disease.
13. **Norman W. Karr,** *University of California Medical School*
    The eserine-like action of certain new urethane methiodides.
14. **R. L. Landau and A. D. Welch,** *Washington University School of Medicine, St. Louis*
    The arsenic analogue of choline as a component of lecithin in rats fed arsenocholine chloride.
15. **C. D. Leake,** *University of California Medical School*
    The estimation of the safety margin of inhalation agents.
16. **A. J. Lehman and H. F. Chase,** *Wayne University College of Medicine, Detroit*
    The curare-like action of certain new synthetic agents.
17. **R. C. Li,** *Peiping Union Medical College*
    Tolerance to repeated doses of acetins in rats and mice.
18. **David Fielding Marsh,** *University of California Medical School*
    Biochemorphology of the symmetrical ethers.
19. **Elton L. McCawley,** *University of California Medical School*
    The toxicity of 1,2-dichloroethane, 2-chloro-2-butene and 1,2,3-trichlorobutane.
20. **A. R. McIntyre and Ray E. King,** *University of Nebraska College of Medicine*
    Polarographic investigation of local anesthetics.
21. **Barbara McIvor, Hamilton H. Anderson, F. P. Luduena and C. D. Leake,** *University of California Medical School and Peiping Union Medical College*
    Chemotherapeutic effectiveness of certain sulfonamide, organic antimony, arsenic, and bismuth compounds in experimental Chagas' disease.
22. **Shih-Yi P'an and Ch'ing-Shan Lu,** *Peiping Union Medical College*
    Effect of a monopiperidine biuret and other hypnotics on the oxygen consumption of rat liver.
23. **George J. Pastorius,** *University of Pittsburgh School of Medicine*
    The pharmacological action of hydroxyethyl-apocupreine on some isolated tissues.
24. **Charles Pecher, John H. Lawrence and Joseph G. Hamilton,** *University of California Radiation Laboratory and Medical School*
    Biological investigations with radioactive calcium and strontium.
25. **James M. Shaffer,** *University of Minnesota Medical School, Minneapolis*
    Enzymatic deacetylation of acetylsulfonamide compounds.
26. **F. E. Shideman and J. K. Theisen,** *University of Wisconsin*
    The oxygen consumption of cerebrum from chronically morphinized rats.
27. **Herbert Silvette,** *University of Virginia*
    Effect of alcohol on post-pituitary antidiuresis.
28. **Mayo H. Soley and Nathan W. Shock,** *University of California Medical School*
    Effect of lowered oxygen tension of inspired air on the respiratory response of normal subjects to carbon dioxide.
29. **H. Y. Soong and S. C. Fu,** *Peiping Union Medical College*
    Toxic range and reaction of 4,4′ diamidino stilbene and p-tolamidine hydrochloride.
30. **Sergei V. Spiridonoff,** *University of California Medical School*
    Public health hazard of certain resins proposed for fruit coatings.
31. **Joseph B. Swim, Elton L. McCawley and Louis A. Strait,** *University of California Medical School*
    Potential industrial hazard from "mouthing" galvanized nails.
32. **John A. Vaichulis,** *Loyola University School of Medicine*
    The effect of some organic solvents on the hormones of the posterior lobe of the hypophysis.
33. **J. A. Wells and T. Mercer,** *Northwestern University Medical School*

The effect of autonomic drugs on the contraction of the colon produced by pelvic nerve stimulation.

34. **Frederick F. Yonkman, A. J. Lehman and H. F. Chase,** *Wayne University College of Medicine, Detroit*
Propylene glycol as a solvent for sulfonamides.

35. **Fredrick F. Yonkman and Clarence Walton,** *Wayne University College of Medicine Detroit*
Blood levels of sulfanilamide following intraperitoneal injection of a 10% solution of sulfanilamide in propylene glycol.

PATHOLOGY

Thursday, 2:00 p.m.

HAWTHORNE ROOM

Joint Meeting with the American Association of Immunologists

1. **Harry Eagle and Morris Rosenfeld** (*by invitation*), *United States Public Health Service and Johns Hopkins Medical School*
Some ultracentrifugal properties of complement. (Path.)

2. **Michael Heidelberger and Otto G. Bier** (São Paulo) (*by invitation*) **and Manfred Mayer** (*by invitation*), *Columbia University and the Presbyterian Hospital, N. Y.*
Fixation of complement nitrogen and complement components by specific precipitates. (Immunol. Invitation by Path.)

3. **Henry P. Treffers** (*by invitation*), **Michael Heidelberger and Jules Freund,** *Columbia University and the Presbyterian Hospital, New York, and the Research and Antitoxic Laboratory, New York City Department of Health, Otisville, N. Y.*
Protein-antiprotein reactions in horse sera. (Immunol. Invitation by Path.)

4. **Francis C. Lowell** (*Introduced by* Charles F. Branch), *Evans Memorial, Massachusetts Memorial Hospitals and Boston University*
A comparison of the collodion particle technique with other methods of measuring antibody. (Path.)

5. **Douglas H. Sprunt and Haywood Taylor** (*by invitation*), *Duke University*
The influence of edema on the susceptibility of the rabbit to infection with vaccinia. (Path.)

6. **C. Dale Barrett, Jr. and John T. Tripp** (*by invitation*), *Michigan Department of Health Laboratories, Lansing, Michigan.*
A method for adjusting the antigen-antibody ratio prior to the determination of pneumococcic antibody nitrogen. (Immunol. Invitation by Path.)

7. **O. Saphir, M. Appel** (*by invitation*) **and M. Janota** (*by invitation*), *Michael Reese Hospital, Chicago*
Complement fixing antibodies (Brown-Pearce carcinoma) in the blood serum and in the aqueous fluid of the anterior chamber of the eye. (Path.)

8. **Monroe D. Eaton and Walter P. Martin** (*by invitation*), *California State Department of Public Health, Berkeley*
The relation of antibodies in human beings to immunity and infection with influenza A. (Immunol. Invitation by Path.)

9. **G. M. Kalmanson** (*by invitation*) **and J. Bronfenbrenner,** *Washington University*
The reactivation of neutralized phage and other biologic agents by digestion of the antibody with papain. (Path.)

10. **Henry W. Scherp, George P. Berry and Dorothy R. Shaw** (*by invitation*), *University of Rochester School of Medicine and Dentistry*
Some immunochemical aspects of the fibroma-myxoma transformation. (Immunol. Invitation by Path.)

11. **Sol Roy Rosenthal,** *University of Illinois*
On a method of decreasing the reactivity to tuberculin by the inhalation of fumes from a boiling suspension of tubercle bacilli. (Path.)

12. **C. M. McKee and E. Alture-Werber** (*by invitation*), *Squibb Institute for Medical Research, New Brunswick*
Use of the chick embryo for evaluation of the efficacy of drugs in tuberculosis. (Immunol. Invitation by Path.)

13. **George Hartley, Jr.,** *Evans Memorial, Massachusetts Memorial Hospitals and Boston University*
Experimental allergic inflammation of the intestine. (Path.)

14. **Philip Levine,** *Newark Beth Israel Hospital, Newark, New Jersey*
The Rh factor in the pathogenesis of erythroblastosis fetalis (Immunol. Invitation by Path.)

PATHOLOGY

Friday, 9:00 a.m.

HAWTHORNE ROOM

Vitamins: Microbiology

1. **M. A. Blankenhorn, Maurice Landy** (*by invitation*) **and Jane Blankenhorn** (*by invitation*), *University of Cincinnati and the Research Laboratories of the S.M.A. Corporation*
The effect of a deficiency of vitamin A on ciliary motion in rat's trachea.

2. **Ernst Simonson, Albert Baer** (*by invitation*) **and Norbert Enzer** (*by invitation*), *Mt. Sinai Hospital and the Lakeside Laboratories, Milwaukee*
   The influence of vitamin B (complex) surplus on the capacity for muscular and mental work. (Physiol.)
3. **Walter W. Wainio** (*introduced by* E. B. Forbes), *Institute of Animal Nutrition, State College, Pennsylvania*
   The thiamine requirement of the albino rat as related to the carbohydrate, protein and fat of the diet. (Physiol.)
4. **M. M. Wintrobe, M. H. Miller** (*by invitation*) **and R. H. Follis, Jr.** (*by invitation*), *Johns Hopkins University*
   The effect of vitamins, especially pyridoxine and pantothenic acid, in preventing sensory neuron degeneration due to nutritional deficiency in pigs.
5. **Karl H. Beyer** (*introduced by* Walter J. Meek), *University of Wisconsin*
   The protective action of vitamin C against experimental liver damage. (Physiol.)
6. **C. I. Reed, H. C. Struck and B. P. Reed** (*by invitation*), *University of Illinois, Chicago*
   A comparative study of normal, rachitic and healed rachitic bone by x-ray diffraction technic. (Physiol.)
7. **Evan W. McChesney** (*introduced by* O. W. Barlow) *Research Laboratories, Winthrop Chemical Company*
   A critique of various methods for the bioassay of dihydrotachysterol (A.T. 10). (Physiol.)
8. **Anna M. Baetjer and Frederick J. Vintinner** (*by invitation*), *Johns Hopkins School of Hygiene and Public Health*
   The effect of dust inhalation on susceptibility to pneumonia (Physiol.)
9. **Geoffrey Rake and Helen Jones** (*introduced by* Morris E. Shaffer), *The Squibb Institute for Medical Research*
   Chemotherapy and persistent infection in lymphogranuloma venereum.
10. **Roger Denio Baker,** *Duke University*
    Chronic progressive sporotrichosis in mice.
11. **E. C. Rosenow,** *Mayo Foundation*
    Poliomyelitis produced directly in monkeys with virus derived from "neurotropic" streptococci.
12. **Morris F. Shaffer, Geoffrey Rake** (*by invitation*), **Joseph Stokes, Jr.** (*by invitation*) **and Elizabeth P. Maris** (*by invitation*), *The Squibb Institute for Medical Research*
    Results following inoculation of egg-passaged measles virus into children by various routes.

PATHOLOGY A

Friday, 2:00 p.m.

HAWTHORNE ROOM

Hematology

1. **W. T. Pommerenke** (*introduced by* P. F. Hahn), *University of Rochester*
   The passage of radioactive iron through the human placenta.
2. **William M. Balfour** (*introduced by* P. F. Hahn), *University of Rochester*
   The absorption of radioactive iron in human beings.
3. **Milan A. Chapin** (*by invitation*) **and Joseph F. Ross,** *Evans Memorial, Massachusetts Memorial Hospitals, and Boston University School of Medicine.*
   Factors influencing the metabolism of radioactive iron in human subjects.
4. **Joseph F. Ross and Milan A. Chapin** (*by invitation*), *Evans Memorial, Massachusetts Memorial Hospitals, and Boston University School of Medicine*
   The direct determination of erythrocyte volume in human subjects with radioactively tagged cells.
5. **P. F. Hahn,** *University of Rochester*
   Circulating red cell mass as a linear function of the venous hematocrit in the dog.
6. **E. T. Mertz** (*introduced by* A. G. Hogan), *University of Missouri*
   Studies on inherited bleeding disease in swine. (Biochem.)
7. **Leandro M. Tocantins,** *Jefferson Medical College*
   Antithromboplastic activity of normal and hemophilic plasmas. (Physiol).
8. **A. J. Quick,** *Marquette University*
   Comparison of the action of Russel viper venom with that of thromboplastin. (Biochem.)
9. **Walter H. Seegers** (*introduced by* H. P. Smith), *State University of Iowa*
   Further purification of thrombin: solubility curve of purified products.
10. **O. M. Gruhzit,** *Parke, Davis and Company*
    Vitamin K anemia in experimental animals.
11. **C. A. Baumann, J. B. Field** (*by invitation*), **R. S. Overman** (*by invitation*) **and K. P. Link,** *University of Wisconsin*
    Induced hypoprothrombinemia and vitamin C excretion. (Biochem.)
12. **Paul L. McLain, George J. Pastorius and C. H. William Ruhe** (*introduced by* C. C. Guthrie), *University of Pittsburgh*
    The effect of anticoagulants on the specific gravity of blood and plasma. (Physiol.)

13. **Edna H. Tompkins,** *Vanderbilt University*
Lecithin intravenously: its relationship to the lipoid storage diseases and to the hemolytic anemias.
14. **Eric Ponder,** *Nassau Hospital, Mineola, Long Island, N. Y.*
The disk-sphere transformation of red cells by lecithin. (Physiol.)
15. **Hugh Davson,** *Dalhousie University*
Potassium salt haemolysis. (Physiol.)

PATHOLOGY B

Friday, 2:00 p.m.

See Notice for Location

Neoplasms and Hyperplasia

1. **Robert J. Stein** (*introduced by* H. P. Smith), *Orleans County Memorial Hospital, Newport, Vt.*
Reticulum fiber-lymphocyte response in malignant neoplasms.
2. **Paul E. Steiner,** *University of Chicago*
The comparative pathology of induced tumors of the salivary glands.
3. **Edward L. Burns and John R. Schenken** (*by invitation*), *Louisiana State University*
Spontaneous primary hepatomas in mice of strain C3H. II. The effect of estrogens and testosterone on the incidence.
4. **Lloyd C. Fogg** (*by invitation*) **and Shields Warren,** *New England Deaconess Hospital*
Effect of repeated doses of radiation of mouse sarcoma 180 on abnormalities of mitosis.
5. **Herman Lisco** (*by invitation*) **and Shields Warren,** *New England Deaconess Hospital*
The changes in blood count associated with radiations from the cyclotron.
6. **Charles F. Branch,** *Boston University*
The mitotic activity of a group of cholchicine-like compounds.
7. **Shields Warren,** *New England Deaconess Hospital*
The distribution of radioactive phosphorus administered to leukemic patients.
8. **H. Hirschmann** (*introduced by* Carl Bachman), *University of Pennsylvania.*
Isolation of a new triol from the urine of a patient with an adrenocortical carcinoma. (Biochem.)
9. **B. Lucké and H. Schlumberger** (*by invitation*), *University of Pennsylvania*
The effects of temperature on growth and metastasis of spontaneous frog carcinoma.
10. **H. Schlumberger** (*by invitation*) **and B. Lucké,** *University of Pennsylvania*
Intraocular transplantation of frog carcinoma through 15 serial generations.
11. **Dale Rex Coman,** *University of Pennsylvania*
Human neoplasms in tissue culture.
12. **Isolde T. Zeckwer,** *University of Pennsylvania*
Mammary gland hyperplasia in rats united in parabiosis with gonadectomized partners.

PATHOLOGY

Saturday, 9:00 a.m.

Hawthorne Room

Kidney and Circulatory System

Unclassified Topics

1. **Norman M. Keith, Harry E. King** (*by invitation*) **and Arnold E. Osterberg** (*by invitation*), *Mayo Clinic*
The serum concentration and renal clearance of potassium in severe renal insufficiency.
2. **Benjamin Jablons** (*introduced by* Frederick M. Allen), *Polyclinic, Welfare and City Hospitals, New York City*
Effect of vasoactive preparations on renal circulation of exteriorized kidneys.
3. **Frederick M. Allen and William S. Youland** (*by invitation*), *New York Medical College*
Experiments with circulatory alterations in explanted kidneys.
4. **Cyrus C. Erickson,** *Duke University*
Effects of pregnancy on blood pressure in chronic hypertensive and normal dogs.
5. **Rudolph Mrazek** (*by invitation*), **Clarence G. Novak** (*by invitation*) **and C. I. Reed,** *University of Illinois*
Further study of the influence of activated sterols on blood pressure. (Physiol.)
6. **Russell L. Holman,** *University of North Carolina*
Experimental arterial lesions related to diet and renal insufficiency.
7. **L. Friedberg and M. Hurwitz** (*introduced by* L. N. Katz), *Michael Reese Hospital*
Cardiac hypertrophy in experimental atheromatosis and its relation to the cholesterol content of the heart. (Physiol.)
8. **C. R. Linegar, Theodore Koppanyi and Melvin W. Green** (*by invitation*), *Georgetown University*
The intraureteral injection of drugs into the renal pelvis of the dog. (Pharmacology)
9. **Carl W. Walter and Reuben Z. Schulz** (*introduced by* Elliott Cutler), *Harvard Medical School*
Animal tissue response to metallic magnesium and its alloys.
10. **George M. Hass,** *Cornell Medical College*
The isolation of a polysaccharide from amyloid-bearing tissues.

11. **Stephen Maddock, Gerhardt Schmidt** (*by invitation*) **and Sigfried J. Thannhauser** (*by invitation*), *Boston City Hospital and the Boston Dispensary*
The effect of the intravenous injection of alkaline intestinal phosphatase on dogs following hepatectomy and total abdominal evisceration with and without hepatectomy.
12. **Gregory Pincus,** *Clark University*
Nutrition of the rabbit ovum *in vitro*. (Physiol.)
13. **Arthur T. Hertig and John Rock** (*by invitation*), *Free Hospital for Women and the Harvard Medical School*
On a series of 9 human fertilized ova (5 normal and 4 pathological) obtained in surgically removed uteri prior to the first missed menstrual period.

AMERICAN INSTITUTE OF NUTRITION

Wednesday, 9:00 a.m.

Ball Room, Copley-Plaza

I. First Scientific Session

1. **Harry G. Day,** *Indiana University, Bloomington*
The effects of zinc deficiency in the mouse.
2. **William M. McCord and Fred G. Brazda** (*introduced by* H. H. Beard), *School of Medicine, Louisiana State University*
Vitamin content of rice polishings.
3. **Gladys M. Cook** (*by invitation*) **and Helen S. Mitchell,** *Massachusetts State College, Amherst*
The anticataractogenic effect of certain dicarboxylic diamino acids.
4. **George R. Sharpless** (*introduced by* J. M. Orten), *Henry Ford Hospital, Detroit*
Diet and epithelial hyperplasia in the forestomach of rats and mice.
5. **S. M. Small** (*by invitation*) **and A. T. Milhorat,** *Cornell University Medical College and the New York Hospital, New York*
Metabolism in anorexia nervosa.
6. **Joseph I. Routh and O. Boyd Houchin** (*introduced by* H. A. Mattill), *State University of Iowa, Iowa City*
Some nutritional requirements of the hamster.
7. **D. W. Woolley,** *Rockefeller Institute for Medical Research, New York*
Some new dietary essentials required by guinea pigs.
8. **R. C. Mills** (*by invitation*), **G. M. Briggs, Jr.** (*by invitation*), **E. B. Hart and C. A. Elvehjem,** *University of Wisconsin, Madison*
Further studies on L. casei e eluate factor in the nutrition of the chick.
9. **Vivian M. Roberts** (*by invitation*), **and Lydia J. Roberts,** *University of Chicago*
A study of the ascorbic acid requirements of children of early school age.

II. Business Meeting, *11:30 a.m.*

III. Second Scientific Session

Wednesday, 2 p.m.

1. **Philip Handler** (*by invitation*) **and W. J. Dann,** *Duke University School of Medicine, Durham*
Water balance in canine blacktongue.
2. **J. V. Lowry** (*by invitation*), **W. H. Sebrell, F. S. Daft and L. L. Ashburn** (*by invitation*), *National Institute of Health, Bethesda*
Polyneuropathy in thiamine-deficient rats delayed by alcohol or whiskey.
3. **C. G. Mackenzie** (*introduced by* E. V. McCollum), *Johns Hopkins University, Baltimore*
The development of E deficiency in rabbits and the response to alpha-tocopherol as measured by creatine excretion.
4. **Edward Nielson** (*by invitation*) **and C. A. Elvehjem,** *University of Wisconsin, Madison*
Biotin deficiencies in the rat.
5. **Sadie Brenner** (*by invitation*) **and Lydia J. Roberts,** *University of Chicago*
Effect of vitamin A depletion in young adults.
6. **R. D. Williams and H. L. Mason** (*by invitation*), *Mayo Foundation, Rochester*
Observations on induced riboflavin deficiency in man.
7. **K. C. Hamner, C. B. Lyon, G. H. Ellis and K. C. Beeson** (*introduced by* L. A. Maynard), *U. S. Plant, Soil and Nutrition Laboratory, Ithaca*
Factors influencing the nutritive value of the tomato.
8. **R. W. Engel** (*introduced by* W. D. Salmon), *Alabama Polytechnic Institute, Auburn*
Biological and chemical studies related to choline deficiency.
9. **Harold Blumberg, C. G. Mackenzie and David Seligson** (*introduced by* E. V. McCollum), *Johns Hopkins University, Baltimore*
The prevention by choline, methionine or casein of dietary cirrhosis of the liver in rats and rabbits.
10. **F. S. Daft, W. H. Sebrell and R. D. Lillie** (*by invitation*), *National Institute of Health, Bethesda*
The production and prevention of hemorrhagic necrosis and cirrhosis of the liver in rats.

IV. Mead Johnson & Company "B Complex" Award

4:30 p.m.

V. Business Meeting, *5:00 p.m.*

VI. Symposia

8:00 p.m.

A. **Nutrition and the Development of Bones and Teeth**

MONACO ROOM, LENOX HOTEL

Genevieve Stearns, *State University of Iowa,* Chairman

D. M. Greenberg, *University of California*

W. D. Armstrong, *University of Minnesota*

J. C. Aub, *Harvard University*

G. Stearns, *State University of Iowa*

B. **Human Requirements for the Vitamins**

SWISS ROOM, COPLEY-PLAZA

G. R. Cowgill, *Yale University,* Chairman

L. E. Booher, *U. S. Department of Agriculture*

P. C. Jeans, *State University of Iowa*

O. A. Bessey, *Harvard University*

D. Melnick, *Food Research Laboratories, Inc.*

R. D. Williams, *Mayo Foundation*

H. T. Parsons, *University of Wisconsin*

C. A. Elvehjem, *University of Wisconsin*

W. A. Perlzweig, *Duke University*

C. **Relation of Dietary Protein to Formation of Blood Proteins**

SALON, COPLEY-PLAZA

A. G. Hogan, *University of Missouri,* Chairman

S. C. Madden, *University of Rochester*

J. M. Orten, *Wayne University*

E. L. Powell, *University of Missouri*

I. McQuarrie, *University of Minnesota*

D. **Influence of Dehydration on Nutritive Value of Fruits and Vegetables**

DOME ROOM, HOTEL LENOX

L. A. Maynard, *Cornell University,* Chairman

E. M. Mrak, *University of California*

C. Chatfield, *U. S. Department of Agriculture*

P. E. Howe, *U. S. Army*

D. K. Tressler, *New York State Experiment Station*

E. **Fortified Food: Official Standards and Analytical Methods**

FOYER, COPLEY-PLAZA

E. M. Nelson, *Food and Drug Administration,* Chairman

Speaker to be announced

H. K. Stiebeling, *U. S. Department of Agriculture*

E. S. Gordon, *State of Wisconsin General Hospital*

F. C. Bing, *American Medical Association*

B. L. Oser, *Food Research Laboratories, Inc.*

VII. Third Scientific Session

Thursday, 2:00 p.m.

DOME ROOM, HOTEL LENOX

1. **J. L. Bollman,** *Mayo Foundation*
   Thiamine benefits vitamin B-deficient rats receiving only dextrose. (*Pathology*)
2. **Alfred H. Free** (*by invitation*), **Jack R. Leonards** (*by invitation*) **and Victor C. Myers,** *Western Reserve University*
   Studies on the absorption and metabolism of galactose and glycine in dogs with vitamin B deficiency. (*Biochemistry*)
3. **O. Boyd Houchin** (*introduced by* H. A. Mattill), *State University of Iowa*
   Vitamin E and muscle degeneration in the hamster. (*Biochemistry*)
4. **A. E. Schaefer** (*by invitation*), **J. M. McKibbin** (*by invitation*) **and C. A. Elvehjem,** *University of Wisconsin*
   Pantothenic acid deficiency in dogs. (*Biochemistry*)
5. **J. M. Lewis** (*by invitation*), **Oscar Bodansky, K. G. Falk and G. McGuire** (*by invitation*), *Beth Israel Hospital and Laboratory of Industrial Hygiene, New York*
   The relation of vitamin A intake to growth and to concentration of vitamin A in the blood plasma, liver and retina. (*Biochemistry*)
6. **Herbert P. Sarett** (*by invitation*), **J. Raymond Klein and William A. Perlzweig,** *Duke University*
   The effect of variations in protein intake on the urinary excretion of riboflavin, nicotinic acid, pyridoxin and thiamine in dogs. (*Biochemistry*)
7. **John Warkentin** (*introduced by* A. C. Ivy), *Northwestern University*
   A study of dietary self-selection. (*Physiology*)
8. **Mary Adelia Bennett and Gerrit Toennies,** *Lankenau Hospital Research Institute*
   A nutritional assay of oxycasein by growth experiments with albino rats. (*Biochemistry*)
9. **Julius White and Jesse E. Edwards,** *National Institute of Health*
   Effect of oral administration of aniline and p-aminodimethylaniline on the growth of the rat. (*Biochemistry*)
10. **Smith Freeman,** *Northwestern University*
    The effect of aluminum hydroxide ingestion upon iron retention by the anemic rat. (*Physiology*)
11. **Leo T. Samuels, Roger M. Reniecke** (*by invitation*) **and Kenneth L. Bauman** (*by invitation*), *University of Minnesota*

The effect of previous diet on the basal metabolic rate of hypophysectomized and normal male rats. (*Biochemistry*)

12. **F. Pierce Dann, Ruth C. Moore and D. V. Frost** (*introduced by* D. W. MacCorquodale), *Abbott Laboratories*
Nutritional achromotrichia in rats. (*Biochemistry*)

13. **Gustav J. Martin** (*introduced by* Walter Goldfarb), *Warner Institute for Therapeutic Research, New York*
The achromotrichial action of p-aminobenzoic acid. (*Physiology*)

14. **S. Ansbacher, W. A. Wisansky and Gustav J. Martin,** *The Research Laboratory, American Home Products Corp.* (*International Vitamin Corporation Division*), *Richmond Hill, N. Y. and the Warner Institute for Therapeutic Research, New York, N. Y.*
P-aminobenzoic acid and hormones. (*Biochemistry*)

15. **J. M. Orten, G. Sayers,** (*by invitation*), **M. Sayers** (*by invitation*) **and A. U. Orten** (*by invitation*), *Wayne University*
Further studies on the disposition of injected glucose in a diabetic strain of rats. (*Biochemistry*)

16. **Jerome W. Conn** (*by invitation*), **Elizabeth Stern Conn** (*by invitation*) **and Margaret W. Johnston,** *University of Michigan*
Non-utilization versus overproduction of glucose by the depancreatized dog. (*Biochemistry*)

17. **Gordon H. Ellis and L. A. Maynard,** *Cornell University*
Some effects of the level of dietary protein on carbohydrate and protein metabolism. (*Biochemistry*)

VIII. Paper Read by Title

1. **P. B. Pearson and H. Schmitt** (*by invitation*), *Texas Agricultural Experiment Station, Agricultural and Mechanical College of Texas*
The effect of diet on the excretion of pantothenic acid in the urine of equine.

INDEX OF NAMES ON SCIENTIFIC PROGRAMS

A

Abbott, L. DeF., Jr. 19
Abramson, D. I. 12
Abreu, B. E. 23, 26
Adams, M. H. 18
Adams, W. L. 26
Ades, H. W. 11
Adler, H. F. 10
Adolph, E. F. 6
Albanese, A. A. 2, 16, 19
Albert, S. 13
Allen, F. M. 6, 31
Allen, J. G. 9
Allen, W. F. 8
Almquist, H. J. 17
Alt, H. L. 4
Altshuler, C. H. 12
Alture-Werber, E. 29
Amberson, W. R. 4
Ambrose, A. M. 27
Anchel, M. 18
Anderson, C. E. 21, 22
Anderson, H. H. 27, 28
Anderson, R. 14
Anderson, R. C. 24
Andrews, H. L. 23
Andrews, J. C. 21, 22
Angerer, C. A. 14
Angerer, H. 14
Ansbacher, S. 34
Appel, M. 29
Archibald, R. M. 19, 21
Armstrong, W. D. 21, 33
Arnett, V. 8
Artom, C. 18
Ashburn, L. L. 32
Asher, R. 9
Ashman, R. 12
Astwood, E. B. 7
Atcheson, G. H. 5
Atkinson, A. J. 10
Aub, J. C. 33
Auerbach, O. 25
Aumann, K. W. 16
Axelrod, A. E. 21
Axelrod, B. 2

B

Bachman, C. 31
Bachmann, G. 14
Baer, A. 30
Baetjer, A. M. 30
Bailey, J. H. 19
Bair, H. L. 11
Baker, R. D. 30
Baldes, E. J. 12
Balfour, W. M. 30
Balls, A. K. 22
Barborka, C. J. 13
Barbour, J. H. 24
Bard, P. 2, 8, 15
Barkan, G. 2, 23
Barker, S. B. 7
Barlow, O. W. 30
Barnes, B. 7
Barnes, F. W. 19
Barnes, R. H. 17
Barnes, T. C. 11
Barnum, C. P. 21
Barrera, S. E. 3
Barrett, C. D., Jr. 29
Barstow, R. 5
Barton, C. F. 9
Batterman, R. C. 23
Baudisch, O. 20
Bauer, C. D. 22
Bauman, K. L. 33
Baumann, C. A. 30
Baxt, J. O. 8
Beach, E. F. 18
Bean, J. W. 6, 12, 14
Beard, H. H. 2, 19, 32
Becker, R. F. 10, 15
Becker, T. J. 26
Beckman, H. 25, 26
Beeson, K. C. 32
Bennett, H. S. 2
Bennett, M. A. 33
Benning, E. H. 17
Berg, C. P. 17, 22
Berg, R. 17
Bergmann, M. 21
Berkman, S. 19
Bernheim, F. 19
Bernhart, F. W. 16
Berry, G. P. 29
Bessey, O. A. 33
Bessey, O. E. 16
Beuding, E. 7
Beutner, R. 4, 11, 27
Beyer, K. H. 30
Bier, O. G. 29
Bing, F. C. 26, 33
Bing, R. J. 14
Bischoff, F. 18
Blair, H. A. 4
Blair, J. R. 7
Blankenhorn, J. 29
Blankenhorn, M. A. 29
Bloch, H. S. 17
Bloor, W. R. 18, 21
Blum, H. F. 7
Blumberg, H. 32
Boals, R. T. 6
Bodansky, O. 6, 33
Bohr, D. F. 14
Bollman, J. L. 16, 33
Booher, L. E. 33
Borchers, R. 17
Borduas, A. 6
Bourque, J. E. 11
Bowman, D. E. 18
Boyd, E. M. 27
Boyd, M. J. 16
Boyd, T. E. 4
Bozler, E. 6
Brady, J. M. 14
Branch, C. F. 29, 31
Brand, E. 21
Brassfield, C. R. 2, 5, 6
Braun-Menendez, E. 12
Brazda, F. G. 19, 32
Brenner, S. 32
Brewer, G. 6
Briggs, G. M., Jr. 32
Brink, F. 18
Brink, F., Jr. 4
Britton, S. W. 14
Brobeck, J. R. 7, 8
Brodie, B. B. 22
Bronfenbrenner, J. 29
Bronk, D. W. 4, 18
Brooks, C. 25, 26
Brooks, C. McC. 8, 15
Browne, J. S. L. 13, 17
Bruce, F. 27
Bruger, M. 22
Bruhn, J. M. 9
Brush, B. 6
Bucher, G. R. 14
Buchter, J. A. 26
Bueding, E. 17
Burns, E. L. 31
Burr, G. O. 2, 19
Burrill, M. W. 14
Buschke, W. 2
Butts, J. S. 17

C

Cahill, W. M. 19
Cain, C. K. 17
Calesnick, B. 27
Calkins, V. P. 22
Campbell, B. 7
Campbell, J. 27
Campbell, W. W. 22
Canavarro, K. deS. 8
Cannon, W. B. 7
Cantarow, A. 13, 14
Canzanelli, A. 4
Carlson, A. J. 13
Carlson, G. R. 3
Carmichael, E. B. 14, 15, 25
Carr, C. J. 24
Cassels, D. E. 4
Cattell, McK. 25
Chaffee, E. 17
Chambers, R. 2, 12
Chambers, W. B. 7
Chapin, M. A. 30
Charipper, H. A. 5, 14
Chase, H. F. 23, 28, 29
Chatfield, C. 33
Chen, K. K. 24, 25
Childs, A. 20
Chin, Y. C. 28
Chow, B. F. 5
Ch'uan, T. K. 28
Chunn, C. F. 6
Ciereszko, L. S. 18
Clark, B. B. 24, 26
Clark, D. B. 5
Clark, W. M. 17
Clarke, E. 13
Clarke, G. J. 18
Clausen, S. W. 20
Climenko, D. R. 22
Code, C. F. 4, 10
Coffey, H. 2
Coffey, W. B. 2
Cohen, A. 27
Cohen, P. 17
Cohen, S. S. 19

Cohn, E. T. 20
Cole, K. S. 3, 4
Cole, V. V. 27
Collins, D. A. 8, 9
Collord, J. 21
Coman, D. R. 31
Comroe, J. H., Jr. 10
Cone, N. M. 24
Conklin. R. E. 6, 9
Conn, E. S. 34
Conn, J. W. 34
Cook, G. M. 32
Coolidge, T. B. 19, 22
Coombs, H. C. 14, 28
Cooper, G. R. 21
Cope, O. M. 6
Corbin, K. B. 15
Corcoran, A. C. 12, 14
Cori, C. F. 16
Cornatzer, W. E. 22
Cortell, R. 24
Coryell, C. D. 16
Cosby, R. S. 2, 8, 15
Cox, W. M., Jr. 19
Cowgill, G. R. 20, 33
Crandall, L. A., Jr. 6
Csonka, F. A. 19
Cullen, M. L. 8
Culler, E. A. 8, 11
Cunningham, R. W. 23
Curtis, G. M. 5
Cutler, E. 31

D

D'Angelo, S. A. 5
Daft, F. S. 32
Dahlstet, J. 25
Dann, F. P. 34
Dann, W. J. 32
Darrow, C. W. 3
Davenport, H. W. 13
David, N. A. 26
Davies, D. F. 22
Davies, P. W. 4
Davis, H. 11, 12
Davis, J. E. 14
Davson, H. 31
Dawson, A. B. 13
Dawson, P. M. 4
Day, H. G. 32
Deeley, S. E. 2
DeGraff, A. C. 11, 25
Deichmann, W. 24
DeMarsh, Q. B. 4
de Espanes, E. M. 25
Deuel, H. J., Jr. 18
Dille, J. M. 25
Dillon, J. B. 12
DiPalma, J. R. 12
Dische, Z. 16
Doisy, E. A. 2, 17
Donahue, D. D. 24
Dorfman, A. 19
Dosne, C. 6, 7
Dotti, L. B. 10
Dow, P. 3
Dow, R. S. 14
Dragstedt, C. A. 28
Dragstedt, L. R. 6, 9
Draize, J. H. 24
Dresbach, M. 25
Dripps, R. 24
Drips, D. G. 20
Driver, R. L. 11
Drury, D. R. 9
Dukes, H. H. 15
Dumke, P. 24
Dumke, P. R. 10
Dunn, M. S. 16
Du Vigneaud, V. 19
Dworkin, S. 8
Dye, J. A. 7
Dyniewicz, H. 27

E

Eagle, H. 24, 29
Eaton, M. D. 29
Eckstein, R. W. 2
Eddy, N. B. 23
Edwards, J. E. 33
Eichelberger, L. 20
Eichna, L. W. 25
Elderfield, R. C. 25
Elgart, S. 9
Elliott, K. A. C. 19
Ellis, G. H. 32, 34
Ellis, S. 23
Elvehjem, C. A. 21, 26, 32, 33
Emerson, G. A. 24
Engel, R. W. 32
Ensor, C. 3, 14
Enzer, N. 30
Erickson, C. C. 31
Erickson, J. O. 21
Escobar, R. A. 26
Espenen, J. K. 2
Essex, H. E. 12
Ets, H. N. 5
Ettinger, G. H. 26
Evans, E. A., Jr. 19
Evans, W. E., Jr. 23
Eversole, W. J. 5
Eyster, J. A. E. 6

F

Falk, K. G. 33
Fassett, D. W. 11
Fazekas, J. F. 5, 17
Feindel, W. H. 11
Feinstein, R. N. 9
Feitelberg, S. 2
Felder, R. E. 11
Fellows, E. J. 23
Fenn, W. O. 12
Field, J., 2nd. 4
Field, J. B. 30
Fierst, S. M. 12
Finnegan, J. K. 28
Fisher, A. M. 21
Fisher, K. C. 4
Fleischmann, W. 5
Flacks, K. 12
Flexner, J. 22
Flexner, L. B. 14
Flock, E. V. 16
Floyd, N. F. 16
Fogg, L. C. 31
Folch, J. 18
Follis, R. H., Jr. 2, 30
Foltz, E. 13
Forbes, E. B. 30
Forbes, J. C. 19
Ford, Z., Jr. 21
Forman, S. E. 24
Fournier, G. 27
Fraser, T. A. 15
Free, A. H. 22, 33
Freeman, L. W. 4
Freeman, N. E. 8
Freeman, S. 6, 11, 33
French, C. R. 14
Freund, J. 29
Friedberg, L. 31
Friedemann, T. E. 22
Frieden, E. H. 16
Friedgood, H. B. 7
Friedman, M. M. 22
Fries, E. C. 2
Frost, D. V. 34
Fruton, J. S. 21
Fu, S. C. 28
Fuerstner, P. G. 28
Fuhrman, F. A. 4
Fulton, G. P. 2
Fulton, J. F. 10, 15
Furchgott, R. F. 6
Futcher, P. H. 19

G

Gaebler, O. H. 2
Gaffron, H. 19
Galambos, R. 11
Gales, J. W. 23
Gantt, W. H. 8
Gardner, J. 21
Gaunt, R. 5
Gavin, G. 21
Geiling, E. M. K. 24
Gellhorn, A. 14
Gellhorn, E. 3, 8, 14
Gemmill, C. L. 10
Gerard, R. W. 3, 5, 18
Gesell, R. 2, 5, 6
Gibbs, E. L. 3
Gibbs, F. A. 3
Gibson, R. B. 17, 22
Gildea, E. F. 20
Gilson, L. E. 22
Gilson, W. E. 6
Girden, E. 8
Glaser, G. H. 8
Godfrid, M. 17
Goebel, W. F. 18
Gold, H. 23, 25
Goldfarb, W. 7, 34
Goodman, H. C. 11
Gordon, A. S. 5, 14
Gordon, E. S. 33
Goudsmit, A. 6
Grady, H. G. 7
Graef, I. 3, 7
Graham, J. 3
Graubard, M. 13
Gray, J. S. 10, 13, 14
Grayman, I. 9
Green, D. E. 16
Green, H. D. 2, 8, 14
Green, M. W. 25, 31
Greenberg, D. M. 20, 33
Greene, R. R. 14
Greengard, H. 4, 11, 14
Greenstein, J. P. 21
Greenwald, I. 16, 22
Greep, R. O. 5
Gregersen, M. I. 7, 8
Gregg, D. E. 2, 11, 12
Gregory, R. A. 10
Greiber, M. F. 24
Greisheimer, E. M. 27
Griswold, R. A. 27
Grollman, A. 12
Gross, E. G. 24
Gross, J. 8
Gruhzit, O. M. 30
Grundfest, H. 7
Gunning, R. E. 3
Gurchot 28
Gurin, S. 19
Gustafson, G. E. 22
Guthrie, C. C. 30

H

Haag, H. B. 26
Hadidian, Z. 26
Haege, L. F. 12
Hafkesbring, R. 27
Hahn, P. F. 30
Hailman, H. 3
Haldi, J. 14
Hall, F. G. 10
Hallenbeck, G. A. 10
Halliday, D. 16
Hamilton, A. S. 8, 9
Hamilton, J. G. 28
Hamilton, P. 16
Hamilton, P. B. 19
Hamilton, P. G. 13
Hamilton, W. F. 3, 26
Hamner, K. C. 32
Handler, P. 32
Handley, C. A. 23
Hanke, M. E. 19
Hansen, E. T. 2, 5, 6
Hardwicke, H. M. 4
Hargar, R. N. 18
Harkins, H. N. 6
Harkness, D. M. 20
Harned, B. K. 27
Harris, A. S. 6
Harris, J. S. 27
Harris, P. N. 24
Harris, S. C. 14
Harrison, T. R. 12
Hart, E. B. 32
Hart, W. M. 11
Hartley, G., Jr. 29
Hartman, C. G. 13
Hartman, F. A. 14
Harvey, E. N. 12
Hass, G. M. 31
Haugen, G. E. 22
Hayes, G. J. 2, 12
Heggen, G. 20
Heidelberger, M. 29
Heilbrunn, L. V. 13
Heinen, J. H. 5
Hellebrandt, F. A. 2, 4
Helmer, O. M. 14, 17, 22
Helmholz, H. F., Jr. 9
Hemingway, A. 17, 19
Henny, G. 8
Henny, G. C. 18
Herbst, R. M. 17
Herrick, J. F. 4, 12
Herrin, R. C. 11
Herrlich, H. 5
Herrmann, J. B. 23
Hertig, A. T. 32
Hertzman, A. B. 12
Hess, W. C. 16
Hildebrand, G. J. 12
Hill, H. C., Jr. 13
Himmelsbach, C. K. 23
Himwich, H. E. 5, 17
Hine, C. J. 24
Hines, H. M. 11, 12
Hirschfelder, A. D. 27
Hirschmann, H. 31
Hisey, A. 4
Hitchcock, D. I. 20
Hitchings, G. H. 20
Höber, R. 13
Hodes, R. 9
Hodge, H. C. 18
Hoefer, P. F. A. 3
Hoehn, W. M. 17
Hoff, H. E. 3, 6, 7
Hoffman, M. M. 13, 17
Hoffman, W. S. 22
Hogan, A. G. 30, 33
Hogan, R. B. 24
Holck, H. G. O. 25
Holland, C. G. 14
Hollander, F. 10, 13, 22
Holman, J. H. 24
Holman, R. L. 31
Holt, J. P. 4
Holt, L. E., Jr. 2, 3, 16, 19
Hong, J. 10
Horwitt, M. K. 18, 21
Houchin, O. B. 32, 33
Hough, V. H. 6
Howard, H. W. 16
Howe, P. E. 33
Huggins, C. 5
Hughes, H. B. 17
Hurwitz, M. 31

I

Ice, G. 27
Ingelfinger, F. J. 10
Irvin, J. L. 17
Ivy, A. C. 4, 10, 11, 13, 33

J

Jablons, B. 31
Jacobs, J. 27
Jacobs, J. E. 4
Jacobson, E. 25
Jahn, T. L. 11
Jailer, J. W. 17
Janota, M. 29
Jeans, P. C. 33
Jemerin, E. E. 10, 22
Jochim, K. 3
Johnson, C. A. 12
Johnson, J. F. 26
Johnson, V. 4
Johnston, C. G. 10
Johnston, M. W. 34
Jolliffe, N. 26
Jones, H. 30
Joseph, M. 28
Jung, F. T. 14, 15

K

Kabat, E. A. 18
Kabat, H. 15
Kajdi, C. 16, 19
Kalckar, H. M. 22
Kalmanson, G. M. 29
Kamin, H. 21
Karpovich, P. V. 7
Karr, N. W. 28
Karstens, A. I. 16
Kassell, B. 21
Katz, G. 23
Katz, L. N. 3, 7, 9, 31
Katz, Y. J. 19
Katzman, P. A. 17
Kaufman, W. 6
Kaulbersz, J. 10, 11
Kay, F. A. 14, 15
Keith, N. M. 31
Keller, A. D. 7, 9
Kemmerer, K. S. 19
Kempf, G. F. 17
Kempner, W. 27
Kendall, E. C. 5, 17, 22
Kent, J. D. 2
Kenyon, A. T. 26
Kepler, E. J. 20
Kessler, M. 3, 14
Kies, M. W. 22
Killian, S. 9
King, B. G. 4
King, C. G. 26
King, H. E. 31
King, R. E. 28
Kirby-Smith, J. F. 7
Kitzmiller, K. V. 24
Klaus, R. F. 15

Klein, J. R. 17, 21, 33
Kleinberg, W. 14
Kleiner, I. S. 10
Kline, R. F. 14
Kmieciak, T. C. 22
Knowlton, K. 26
Koch, F. C. 19
Koehler, A. E. 17
Kohlstaedt, K. G. 17, 22
Kohn, H. I. 27
Kolin, A. 11
Kopeloff, L. M. 3
Koppanyi, T. 24, 25, 31
Korr, I. M. 4, 14
Koser, S. A. 19
Kosman, A. J. 11
Kotalik, G. 13
Kozelka, F. L. 24
Krantz, J. C., Jr. 24
Kraus, G. 20
Krayer, O. 23, 25
Krieger, H. P. 11
Krop, S. 25
Kryter, K. D. 11
Kubin, R. 26
Kwit, N. T. 25
Kyker, G. C. 22

L

Lambert, E. F. 8
Lambert, E. H. 14
Lamport, H. 8
Landau, R. L. 28
Landis, C. 11
Landow, H. 18
Landy, M. 29
Langley, L. L. 10
Larrabee, M. G. 9
Larsen, E. M. 2
Larson, P. S. 26
Laug, E. P. 24
Lavie, T. F. 16
Lawrence, J. H. 28
Lawrence, W. E. 9
Lawson, H. 3, 4, 12
Lazere, B. 12
Leake, C. D. 28
Leblond, C. P. 13
Lebond, C. 18
Lee, W. V. 15
Lehman, A. J. 23, 28, 29
Lehman, R. A. 25
Lein, A. 5
Lemon, H. M. 18
Lenox, W. G. 3
Leonard, H. 20
Leonard, W. E. 15
Leonards, J. R. 33
Levine, P. 29
Levine, R. 9
Levine, V. E. 15
Lewis, H. B. 17
Lewis, J. M. 33
Lewis, L. A. 13
Lewis, R. N. 8, 14
Li, R. C. 28
Libet, B. 19
Licklider, J. C. R. 11
Liebling, R. 20
Lillie, R. D. 32
Linegar, C. R. 24, 31
Link, K. P. 30
Lipmann, F. 16
Lipschitz, W. L. 26
Lipscomb, A. 6
Lisco, H. 31
Liston, L. 15
Lloyd, D. P. C. 5
Loew, E. R. 11
Loewe, S. 26
Loewy, A. 4
Logan, M. A. 16, 22
Long, C. N. H. 7, 8
Longenecker, H. E. 18
Longini, J. 4
Louis, L. 6
Lowell, F. C. 29
Lowry, J. V. 32
Lowy, K. 11
Lu, C.-S. 28
Lucké, B. 31
Luduena, F. P. 28
Ludwick, T. M. 5
Lutz, B. R. 2
Lyon, C. B. 32

M

MacCorquodale, D. W. 34
MacFayden, D. A. 16
Macht, D. I. 15
Macht, M. B. 2, 15
MacKenzie, C. G. 27, 32
MacKenzie, J. B. 27
MacLachlan, P. L. 18
Maclean, A. R. 4
Madden, S. C. 33
Maddock, S. 32
Maes, J. P. 8
Maison, G. 13
Maison, G. L. 23
Majovski, G. J. 26
Man, E. B. 5, 20
Manery, F. D. 8
Mann, F. C. 4, 9, 12
Mann, W. 18
Marchello, A. 4
Maris, E. P. 30
Marmont, G. 3
Marrazzi, A. S. 5, 24
Marsh, D. F. 28
Marsh, D. G. 26
Marshak, A. 16
Marshall, E. K., Jr. 24
Marshall, W. H. 11
Martin, A. W. 4
Martin, G. J. 34
Martin, S. J. 24
Martin, W. P. 29
Mason, A. 2, 5
Mason, H. L. 21, 32
Mason, M. F. 20
Masor, N. A. 4
Masson, G. 13
Mast, S. O. 15
Mathieson, D. R. 25
Mattil, K. F. 18
Mattill, H. A. 20, 22, 32, 33
Mattis, P. A. 27
Maurer, J. 16
Mautz, F. R. 20
Mayer, M. 29
Mayerson, H. S. 8
Maynard, L. A. 32, 33, 34
McCawley, E. L. 28
McChesney, E. W. 30
McCollum, E. V. 27, 32
McCoord, A. B. 20
McCord, W. M. 32
McCoy, R. H. 19
McCullach, D. R. 13
McCullagh, D. R. 16
McCullagh, E. P. 13
McGuigan, H. 27
McGuire, G. 33
McHenry, E. W. 21
McIntire, F. C. 17
McIntyre, A. R. 28
McIvor, B. 28
McKee, C. M. 29
McKibbin, J. M. 33
McLain, P. L. 26, 30
McMeekin, T. L. 21
McPhail, M. K. 26
McQuarrie, I. 33
Medes, G. 16
Meek, W. J. 6, 15, 30
Mehl, J. W. 18
Mellish, C. H. 26
Mellors, R. C. 20
Melnick, D. 33
Member, S. 22
Mendenhall, W. L. 3, 23
Mendez, R. 25
Mercer, T. 28
Merlis, J. K. 15
Mertz, E. T. 30
Meschan, I. 10
Mettler, F. A. 3, 8, 15
Meyer, K. 17
Miley, G. 4
Milhorat, A. T. 32
Miller, A. E. 19
Miller, B. F. 18
Miller, J. W. 24
Miller, M. H. 30
Miller, Z. B. 18
Millman, N. 7
Mills, R. C. 32
Mirsky, I. A. 9
Mitchell, H. S. 32
Modell, W. 6, 25
Moe, G. K. 4, 25
Moetsch, J. C. 27
Molitor, H. 26
Moore, D. H. 18
Moore, R. C. 34
Moreland, F. B. 20, 22
Morgareidge, K. 21
Morrison, J. L. 24
Morse, M. 4
Moss, R. E. 10
Moss, W. G. 4
Mrak, E. M. 33
Mrazek, R. 31
Mueller, A. J. 19
Mullins, L. J. 12
Munks, B. 18
Muntwyler, E. 20
Murlin, J. R. 2, 9, 11 18
Myers, V. C. 20, 22, 33

N

Nachmanshohn, D. 5
Nadler, J. E. 25
Nahum, L. H. 6
Najjar, V. A. 2, 3
Necheles, H. 8, 11, 13
Nelson, D. 15
Nelson, E. M. 33
Nelson, N. 9
Nelson, W. O. 13
Neurath, H. 21
Nice, L. B. 15
Nicholson, H. C. 10
Nickerson, N. 6
Nielsen, E. 21
Nielson, E. 32
Nier, A. O. 17, 19
Nims, L. F. 3
Nixon, E. N. 9
Novak, C. G. 31
Nuhfer, P. A. 26

O

Oberst, F. W. 23
Ogden, E. 12
Olander, G. A. 12
Olcott, H. S. 21
Olson, W. H. 8, 11
Opdyke, D. F. 9
Oppenheimer, E. T. 4
Oppenheimer, M. J. 8
Orten, A. U. 34
Orten, J. M. 32, 33, 34
Orth, O. S. 15
Oser, B. L. 33
Oster, R. H. 3
Osterberg, A. E. 20, 31
Otto, H. 25
Overman, R. S. 30
Owens, F. M., Jr. 9

P

Pace, D. M. 15
Pacella, B. L. 3
Paff, G. H. 25
Page, E. W. 12
Page, I. H. 12, 14, 17, 22
Pallares, D. S. 6
P'an, S.-Y. 28
Pappenheimer, J. R. 8
Park, E. A. 2
Parsons, H. T. 21, 33
Paschkis, K. 13
Paschkis, K. E. 5
Pastorius, G. A. 26
Pastorius, G. J. 28, 30
Patras, M. C. 4
Patterson, T. L. 10
Patton, H. D. 8
Paul, W. D. 22
Pearlman, W. H. 13
Pearson, P. B. 34
Pecher, C. 28
Peoples, S. A. 25
Perkins, P. 22
Perlow, S. 9
Perlzweig, W. A. 19, 22, 33
Peters, R. 20
Petersen, W. E. 5
Peterson, W. H. 17, 21
Pfeiffer, C. 23
Phatak, N. 26
Philips, F. S. 22
Phillips, G. W. 14, 15
Phillips, R. A. 19
Pick, E. P. 2
Pike, F. H. 28
Pincus, G. 13, 32
Pitts, R. F. 5
Pizzolato, P. 2
Plummer, A. J. 23
Polskin, L. J. 20
Pommerenke, W. T. 30
Ponder, E. 31
Pool, J. L. 3
Popper, H. 27
Powell, E. L. 33
Power, M. H. 20, 22
Pratt, F. H. 10
Pritchard, W. H. 2, 11, 12
Purdy, M. 27

Q

Quick, A. J. 30
Quigley, J. P. 10

R

Racker, E. 15
Radzow, K. H. 2, 10, 14
Raiziss, G. W. 27
Ralli, E. P. 3, 7, 9
Rake, G. 30
Rakoff, A. E. 13
Ramsey, R. W. 12
Randell, E. 8
Rankin, A. D. 15
Rapport, D. 4
Ratnoff, C. D. 5
Ray, G. B. 12
Read, H. C. 26
Read, M. R. 10
Reed, B. P. 30
Reed, C. I. 30, 31
Rehm, W. S. 3
Reinecke, R. M. 22, 33
Remmert, LeM. F. 17
Renshaw, B. 3, 5
Rice, J. C. 19
Rich, E. 5
Richards, R. K. 15, 24
Richardson, A. P. 27
Riddle, O. 9
Riggs, D. S. 5
Riker, A. J. 17
Ring, G. C. 7
Rivera, G. 26
Roback, R. A. 11
Robbie, W. A. 17
Roberts, L. J. 32
Roberts, V. M. 32
Robinson, A. R. 18
Robinson, F. J. 20
Robinson, S. 7
Robinson, T. W. 13
Robscheit-Robbins, F. R. 2
Rocha e Silva, M. 28
Rock, J. 32
Rodbard, S. 7
Rogoff, J. M. 9
Rojas, A. G. 6
Roos, A. 7
Rose, W. C. 17
Rosen, V. H. 8
Rosenberg, S. 9
Rosenblueth, A. 5
Rosenfeld, M. 29
Rosenow, E. C. 30
Rosenthal, S. R. 29
Ross, J. F. 30
Ross, W. F. 21
Roth, G. M. 12
Roth, L. W. 12
Routh, J. I. 32
Rubenstein, B. B. 5
Rubin, J. 19
Rubin, M. A. 3
Rubin, S. H. 9
Ruch, T. C. 8
Rudolph, G. G. 19
Ruhe, C. H. W. 30
Russell, J. A. 9
Ryer, R. 9

S

Sacks, J. 12
Sah, P. P. T. 27
Salit, P. W. 22
Salmon, W. D. 20, 32
Salomon, K. 20
Salter, W. T. 5
Sample, A. B. 22
Samuels, L. T. 9, 13, 33
Sandblom, J. P. 4
Sandiford, I. 26
Sandow, A. 12
Sandweiss, D. J. 10
Saphir, O. 29
Sapirstein, L. A. 21

Sarett, H. P. 33
Sawyer, M. E. M. 26
Sayers, G. 34
Sayers, M. 34
Seager, L. D. 23
Sealock, R. R. 17
Searcy, H. B. 25
Schaefer, A. E. 33
Schecter, A. E. 8
Schein, A. H. 17
Schenken, J. R. 31
Scherp, H. W. 29
Schiffrin, M. J. 10
Schlayer, C. 27
Schlumberger, H. 31
Schlutz, F. W. 4
Schmidt, C. F. 10, 24
Schmidt, C. L. A. 16
Schmidt, G. 32
Schmidt, L. H. 17
Schmitt, F. O. 18
Schmitt, H. 34
Schoenheimer, R. 19
Schroeder, E. F. 11
Schubert, C. 26
Schultze, M. O. 19
Schulz, R. Z. 31
Sebrell, W. H. 26, 32
Seegers, W. H. 30
Seevers, M. H. 23, 24, 26
Segaloff, A. 13
Seligson, D. 32
Selle, R. M. 24
Seltzer, J. I. 2
Selye, H. 6, 7, 13, 25, 27
Severac, M. 27
Sexton, E. L. 18
Scott, D. A. 21
Scott, D. B. McN. 19
Scott, W. W. 5
Scruggs, W. 11
Shaffer, J. M. 28
Shaffer, M. E. 30
Sharp, D. G. 21
Sharpless, G. R. 32
Shaw, D. R. 29
Shea, P. C. 8
Sheard, C. 11, 12
Shedlovsky, T. 18
Shelton, R. S. 26
Sherman, W. C. 20
Shettles, L. B. 19
Shideman, F. E. 23, 28
Shipley, R. E. 2, 11, 12
Shock, N. W. 7, 28
Shorr, E. 6
Shuler, R. H. 3, 25
Shull, G. M. 21
Shumacker, H. B., Jr. 5
Sichel, F. J. M. 12
Silvette, H. 15, 28
Simister, T. H. 9
Simon, B. E. 20
Simon, F. P. 18
Simonson, E. 30
Sinclair, R. G. 18
Sklersky, S. 20
Slaughter, D. 23
Sloan, H. 15
Slotin, L. 19
Small, S. M. 32
Smirnow, A. E. 20
Smith, E. L. 12, 25
Smith, H. P. 30, 31
Smith, K. U. 11
Smith, P. K. 3, 7
Smith, R. G. 23
Smith, W. K. 11
Smythe, C. V. 16
Snell, E. E. 16
Snider, R. S. 8
Snodgrass, J. M. 3
Snyder, F. F. 10
Sobel, A. E. 20
Sobotka, H. 16
Solandt, D. Y. 8
Soley, M. H. 28
Somogyi, M. 18
Soong, H. Y. 28
Soskin, S. 9
Spencer, E. C. 15
Sperry, W. M. 3, 17
Spiegel, E. 8
Spiegel-Adolf, M. 8, 18
Spies, T. D. 26
Spiridonoff, S. V. 28
Sprunt, D. H. 29
Stanley, W. M. 19
Stannard, J. N. 13
Stearns, G. 33
Steggerda, F. R. 6
Steigmann, F. 27
Stein, R. J. 31
Steiner, P. E. 31
Steinhardt, J. 21
Stekol, J. A. 19
Stern, K. G. 16, 18, 22
Stewart, W. C. 18
Stickney, J. C. 10
Stiebeling, H. K. 33
Stinger, D. 9
Stokes, J., Jr. 30
Stotz, E. 17
Stowell, A. 8
Stoyanoff, V. A. 17
Strait, L. A. 28
Straus, W. L., Jr. 5
Street, S. F. 12
Strong, F. M. 21
Struck, H. C. 30
Sullivan, M. X. 16
Summers, P. 27
Summerson, W. H. 19
Sunderman, F. W. 3
Sure, B. 21
Svirbely, J. L. 24
Swan, K. C. 22
Swanson, M. 18
Swendseid, M. 19
Swendseid, M. E. 17
Sweet, W. H. 2
Swim, J. B. 28
Szego, C. M. 13

T

Talbot, S. A. 11
Tarver, H. 16
Tashiro, S. 22
Taube, H. 25
Taylor, H. 29
Taylor, J. D. 18
Templeton, R. D. 5
Tepley, L. J. 21
Tepperman, J. 7, 8, 9
Thannhauser, S. J. 32
Thienes, C. H. 24, 26
Thiesen, J. K. 23, 28
Thomas, J. E. 5, 13
Thompson, C. R. 26
Thomson, J. D. 12
Titus, H. W. 19
Tobias, J. M. 5
Tocantins, L. M. 30
Toennies, G. 21, 33
Toman, J. E. P. 3
Tompkins, E. H. 31
Torda, C. 23
Tracy, A. H. 21
Travell, J. 25
Treat, A. E. 8
Treffers, H. P. 29
Tressler, D. K. 33
Tripp, J. T. 29
Truax, F. L. 18
Tum-Suden, C. 7
Turrell, E. S. 7
Tweedy, W. R. 22
Tyler, D. B. 5
Tyslowitz, R. 7
Tytell, A. A. 22

U

Upholt, H. B., Jr. 14

V

Vaichulis, J. A. 28
Valaer, P. J. 24
Van Campen, M. G. 26
Van Dyke, H. B. 5
Van Harreveld, A. 5
Van Liere, E. J. 10
Van Loon, E. J. 24
Van Norman, R. 25
Van Slyke, D. D. 16, 19, 21
Vennesland, B. 19
Vermeulen, G. 9
Vinacke, W. E. 11
Vintinner, F. J. 30
Virtue, R. W. 17
Visscher, M. B. 4, 5, 13
Voegtlin, C. 3
Voeglin, W. L. 4
von Oettingen, W. F. 24

W

Waelsch, H. 18
Wainio, W. W. 30
Wakerlin, G. A. 12
Wakerlin, G. E. 14
Wald, G. 11
Walker, B. S. 21
Wallace, G. B. 5
Walter, C. W. 31
Walton, C. 29
Walzl, E. M. 11
Wang, S. C. 7
Wangerin, D. M. 16, 19
Warkentin, J. 33
Warren, C. O. 19
Warren, M. R. 26
Warren, S. 18, 31
Wastl, H. 24
Waters, R. M. 2
Watt, J. G. 10
Wearn, J. T. 2, 11, 20
Weatherby, J. H. 3
Wedd, A. M. 4
Wégria, R. 6
Weichselbaum, T. E. 18
Weinerman, A. 20
Weinstein, V. A. 10, 22
Weir, E. G. 6
Weir, J. R. 12
Weisberger, A. S. 11
Weld, C. B. 11
Welch, A. D. 27, 28
Welch, C. S. 26
Welch, E. A. 18
Wells, H. S. 3
Wells, J. A. 28
Werch, S. C. 22
Werle, J. M. 8, 10, 15
Wertenberger, G. E. 27
Werthessen, N. T. 13
Westerfeld, W. W. 17
Weston, K. 8
Whatmore, G. B. 25
Wheeler, R. E. 20
White, A. 18
White, J. 33
Whitehorn, W. V. 6
Wiggers, C. J. 8, 15
Wiggers, H. C. 8
Wigginton, C. 15
Wilde, W. S. 12
Wilkins, H. F. 26
Williams, E. G. 23
Williams, J. R., Jr. 12
Williams, R. D. 21, 32, 33
Williams, R. R. 16
Wilson, D. W. 19
Wilson, E. B. 20
Winder, C. V. 10, 26
Windle, W. F. 4, 10, 15
Windsor, E. 17
Winfield, J. M. 10
Winkler, A. W. 3, 7
Winter, H. 13
Wintrobe, M. M. 30
Wisansky, W. A. 34
Wolf, A. V. 6
Wolf, S. 10
Wolff, H. G. 10, 23
Wood, E. H. 4
Woodbury, R. A. 26
Woolley, D. W. 32
Woolsey, C. N. 2, 11, 12
Wortis, J. 4
Worzniak, J. J. 5
Wyman, L. C. 7
Wynn, W. 14

Y

Yonkman, F. F. 27, 29
Youland, W. S. 31
Youmans, W. B. 16

Z

Zeckwer, I. T. 31
Zweifach, B. W. 2

THE AMERICAN PHYSIOLOGICAL SOCIETY

FIFTY-FOURTH ANNUAL MEETING

Boston, Mass., March 31, April 1, 2, 3, 4, 1942

(For possible corrections in any of the following abstracts see the next issue)

Peripheral vascular responses in pregnancy. DAVID I. ABRAMSON, KAMILLO FLACHS (by invitation) and SIDNEY M. FIERST (by invitation). *The May Inst. for Medical Research, The Jewish Hospital, Cincinnati, O.* Since it is generally accepted that there is a significant increase in cardiac output and blood volume in pregnancy, it was considered of interest to determine whether or not the peripheral circulation reflected this change. Blood flow measurements, using the venous occlusion plethysmographic method, were made upon 9 pregnant women at monthly intervals during the period of gestation. Because of the possible mechanical effect of the fetus, the rate of blood flow was determined in the leg as well as in the forearm and hand.

It was found that no significant change took place in the circulation to the forearm and leg during the entire period of gestation, although in a few instances a trend toward an increase in flow was apparent in the last lunar month. The average of all the readings taken during the various stages was no greater than that observed in a control group of non-pregnant women. In the 3 cases in which the blood flow in the hand was studied, the readings in the latter portion of pregnancy were either significantly increased or fell within the upper range of the control figures.

On the basis of the above evidence, it was concluded that the increased blood flow during gestation was for the most part shunted to the placental circulation and that the latter was not augmented at the expense of the blood flow to the extremities.

The primary cortical acoustic area of the monkey and the geniculo-temporal radiation. HARLOW W. ADES and RICHARD E. FELDER (introduced E. A. Culler). *Emory Univ., Ga.* The outline of the primary acoustic projection area on the superior temporal gyrus of rhesus monkeys was determined by oscillograph recording of the response to click stimulation. A lesion was then made in the medial geniculate body of the same side and the cortical area remapped.

In the unoperated animal the responsive (to clicks) area was found to occupy the inner half of the posterior extremity of the superior face of the superior temporal gyrus. The area can be located by, but is not conterminous with, a transverse elevation which is probably comparable with Heschl's convolution in man.

After small lesions in the geniculate, discrete areas of the acoustic cortex were found to lose their responsiveness to stimulation, indicating a close point to point projection from correspondingly limited portions of the homolateral medial geniculate. (Slides.)

Segmental activity of the colon. H. F. ADLER and A. J. ATKINSON (introduced by A. C. Ivy). *Dept. of Physiology and Pharmacology, Northwestern Univ. Medical School, Chicago.* The multiple-balloon system in series has indicated the segmental nature of colon motility in both the unanesthetized dog and man. On occasion, all of the balloon-containing segments may be active or quiet. At other intervals only a single segment may be active, whereas segments 3 to 4 centimeters proximal or distal are quiet, or two widely separated segments may be the only regions of the colon showing motor activity. The most characteristic direction of motility propagation is from more proximal to distal colon, but only a few waves appear to traverse the entire colon. It is not uncommon for waves to arise in the mid-colon of the dog and be propagated orally and distally. Fewer instances of antiperistalsis are seen in the distal colon of man.

When two adjacent colon segments manifest the same general type of activity so that a wave "may" pass from one to the other, the two segments then represent an integrated functional unit for propulsive activity; such coordination provides for propulsion of colon contents.

Stimulating drugs such as prostigmine and physostigmine increase both propulsive and non-propulsive motility. Morphine as another type of stimulating drug characteristically increases non-propulsive motility, but decreases the propulsive. Some antispasmodics (atropine) decrease both types of motor activity. Others, such as "trasentin," seem to act chiefly by changing a spastic or an incoordinated segmental activity into a coordinated activity favoring propulsion.

Diuresis and the recovery of water balance. E. F. ADOLPH. *Dept. of Physiology, Univ. of*

B & Z Library

Rochester School of Medicine and Dentistry, Rochester, N. Y. Rats allegedly show water diuresis though apparently in water deficit, when water is given after previous depletion (Heller, Boyd). Validity of this view depends upon how water balance is appraised.

Rats were tested in water depletions of five kinds: *1*. Deprived of water for 24 hours, dry food ad libitum. *2*. Same, but no food. *3*. Same, but force-fed a usual day's food. *4*. Allowed water, half food. *5*. Placed at 37°C for 3 hours without water or food. Thereafter half of each set (*A*) were given by stomach 5 per cent of their weight of water (less than enough to restore weight except in *3* and *4*); half (*B*) were allowed to drink water. Weight changes, water intakes, and water outputs were measured at half-hourly intervals.

Some diuresis followed each water administration (*B*), the urine being of low specific gravity. Voluntary intake (*A*) also produced detectable water diuresis in some tests after privation. Only half the usual food was eaten by rats while deprived of water; body weight was recovered and kept only when full food was consumed. In each set, no matter how water was obtained, *A* and *B* had the same weight deficit after 3 hours.

Water balance was struck, therefore, at lower body weights; weight is a criterion of water content only among rats that consumed equal amounts of food and were heated alike. When these restrictions are observed, rats are found to be as accurate in recovering their water contents after water deficits, and after water excesses, and after both, as are dog and man.

The gonadotropic effect of various steroid compounds. S. Albert (by invitation) and Hans Selye. *Dept. of Anatomy, McGill Univ., Montreal, Canada.* Experiments in mature albino rats indicate that the atrophy of the testis and accessory sex organs normally produced by chronic estradiol treatment is inhibited by Δ^5-dehydro-*iso*-androsterone, methyl testosterone, testosterone, progesterone, Δ^5-androstene-3(β),17(α)-diol, and Δ^5-pregnene-3(β)-ol-20-one. On the other hand desoxycorticosterone acetate, 21-acetoxy-Δ^5 pregnene-3(β)-ol-20-one, pregnane-3,20-dione, cholesterol and stigmasterol are unable to prevent this atrophy.

It had previously been noted (Nelson and Merckel 1937) that there is no close correlation between the gonad protecting activity of a steroid and its ability to maintain the accessory sex organs of the castrate male ("androgenic" or "testoid" activity). Yet all gonad protecting substances so far observed proved to possess some measure of testoid activity and *vice versa*. The present experimental series revealed a testoid compound (ethinyl testosterone) which is devoid of gonad-protecting action and an active gonadotropic steroid (Δ^5-pregnene-3(β)-ol-20-one) which proved inert as a testoid, when assayed on castrate rats. It appears justified to conclude, therefore, that this type of gonadotropic action is entirely independent of testoid activity.

Another noteworthy phenomenon observed was that compounds causing vaginal cornification in the spayed rat and hence proven to possess "folliculoid" activity (Δ^5-pregnene-3(β)-ol-20-one, Δ^5-androstene-3(β),17(α)-diol) are able to prevent the testis atrophy caused by another folliculoid compound namely, estradiol.

The body growth inhibitory effect of estradiol was prevented in these experiments by a variety of steroids, especially Δ^5-androstene-3(β),17(α)-diol, Δ^5-pregnene-3(β)-ol-20-one, testosterone, Δ^5-dehydro-*iso*-androsterone, methyl testosterone and pregnane-3,20-dione.

The effect of partial pancreatectomy on the blood lipids. J. Garrott Allen (by invitation), Frederick M. Owens, Jr. (by invitation), Dorothy Stinger (by invitation) and Lester R. Dragstedt. *Dept. of Surgery, Univ. of Chicago, Ill.* Five dogs were subjected to partial pancreatectomy in which all but approximately 5 per cent of the pancreas was removed. The remaining pancreatic tissue consisted of a small segment attached to the lesser pancreatic ducts. The animals were observed from 10 to 20 months and were maintained on a diet high in fat and received insulin. Each animal displayed a progressive elevation of the total fasting blood lipids over a period of from two to eight months and then stabilized at a level generally about 50 per cent above the normal post-operative level. The blood lipids in these animals are in sharp contrast to that of the totally pancreatectomized dog where there regularly occurs a sharp reduction in the total fasting lipids.

Cortical irradiation in conditioned differentiation as measured by effects on respiration. William F. Allen. *Dept. of Anatomy, Univ. of Oregon Medical School, Portland.* The results of this study are based on thoracic respiratory tracings taken at the time of recording positive and negative foreleg conditioned reflexes after *correct* conditioned differentiation was obtained from a variety of auditory, olfactory, general cutaneous and optic analysers. Control respiratory tracings before conditioning were as follows: Sound and light showed no effect after the first few tests; olfactory caused inhibition and general cutaneous demonstrated no change, inhibition, or excitation.

After correct conditioned differentiation was established—*Excitation* of respiration ordinarily occurred during the intervals of correct positive conditioned reflex tests. The amount, character and type of excitation varied with the animal and analyser, but was consistent for the same test. *Inhibition* of respiration usually accompanied

correct negative conditioned reflex tests from all excitable and easily inhibited dogs; while the neutral type revealed slight or no inhibition. A rapid succession of alternate negative and positive conditioned tests demonstrated 1, ability to respond correctly to each, and 2, that respiration not only changed correctly but often made the change when in a state of inhibition or excitation. The respiratory tracings recorded during an incorrect negative conditioned test sometimes exhibit an early excitable phase approximating the foreleg movement, which was followed, too late, by a long pronounced inhibitory wave. Likewise, correct positive conditioned reflex records may show short intervals of respiratory excitation accompanying two or more foreleg flexions.

There are apparently three kinds of cortical excitation or inhibition: 1, direct reflex to lower centers; 2, incorrect cortical; 3, correct cortical.

Human hemoglobin solutions as a blood substitute. WILLIAM R. AMBERSON, JOYE E. JACOBS (by invitation) and ALAN HISEY (by invitation). *Dept. of Physiology, School of Medicine, Univ. of Maryland, Baltimore, and Dept. of Biochemistry, George Washington Univ. School of Medicine, Washington.* Sterile evacuated solutions of human hemoglobin, properly balanced and buffered, and kept without refrigeration for as long as three months, have been transfused into cats, with simultaneous removal of equal amounts of normal blood. The animals survive after replacement of as much as 85 per cent of normal blood, without pyrogenic reaction or asphyxial signs. Blood pressures tend to be above normal in the first hours after replacement. Hemoglobinemia and hemoglobinuria terminate in from 36 to 48 hours. No tissue pathology has been noted, but hyperemia of the liver usually persists for several weeks, and cells of the reticulo-endothelial system show hemoglobin inclusions for some time.

Water content of Sartorius of adrenalectomized frogs. CLIFFORD A. ANGERER (introduced by Frank A. Hartman). *Dept. of Physiology, Ohio State Univ., Columbus.* (Read by title.) We have shown that there is an appreciable difference in weight changes of muscles in "balanced" solutions of various depending on the "adect" animals' access to water (Am. J. Physiol. **133:** 197; Anat. Rec. **81:** 93 (Suppl.)). Wet and dry weights were compared for Sartorii removed respectively from four groups of frogs: 1, "uncontrolled adects" maintained in a wet environment thus showing a progressive increase in body weight; 2, "controlled adects" kept in an environment in which the moisture and hence the body weights were kept constant; 3, "renal-damaged" (controls); 4, "normals" (controls). Five days after adrenalectomy, Sartorii from "uncontrolled adects" showed an average water content of 85 per cent after the average body weight had increased 24 per cent as compared with 83 per cent, 82 per cent and 83 per cent for "controlled adects," "normals" and "renal-damaged" muscles respectively. These results show that in "adects" the water is retained by the body. Some of this water is shifted into the muscle tissue possibly to satisfy some change in osmotic equilibrium for these muscles lose weight when placed in normal Ringer's solution, while the control muscles show a slight gain. This muscle-weight increase is not of the same order of increase as the body weight, suggesting that water is taken up by some other tissue in far greater amounts than in muscle. This "water" is undoubtedly contained in far greater degree in the extra-cellular compartment as shown by the relatively strong spurt on pithing.

The effect of dehydration on the viability of adrenal insufficient frogs. CLIFFORD A. ANGERER and HELENA ANGERER (introduced by Frank A. Hartman). *Dept. of Physiology, Ohio State Univ., Columbus.* (Read by title.) While "adect" frogs and to a lesser extent their muscles, at least, imbibe water and retain it, as compared to renal-damaged and "normal" frogs (controls), it was of interest to study the reverse water shift induced by gradual dehydration, in a closed system containing anhydrous $CaCl_2$ and a constant current of air. Experiments were performed on four different groups of *Rana pipiens: 1*, "uncontrolled adects" maintained in a wet environment and thus the body weight progressively increased; *2*, "controlled adects"—body weight maintained constant; *3*, "renal-damaged" (controls), and *4*, "normals" (controls). The end-point employed was the disappearance of integumental reflexes and on immediate autopsy the absence of heart action. Adrenal insufficiency was determined by the failure to perform the "righting reflex" after 3 to 5 attempts. All data was obtained at half-hour intervals. Death ensued at $2\frac{1}{2}$, 3, 4, and 4 hours with a corresponding average weight loss (water loss) of 27, 29, 43 and 47 per cent of the original body weight for, respectively, "controlled adects," "uncontrolled adects," "renal-damaged" and "normal" frogs. The results show that "adect" frogs cannot withstand the stress induced by dehydration even though less body water is removed. The time-rate of water loss was lower in the "adects" ("controlled" or "uncontrolled" body weights) than in the "controls."

Changes in the autonomic centers after repeated electrically induced convulsions. V. ARNETT (by invitation) and E. GELLHORN. *Depts. of Physiology and Psychiatry, Univ. of Illinois College of Medicine, Chicago.* It has been shown by Kessler and Gellhorn (Proc. Soc. Exper. Biol. Med., **46:** pp. 64–66, 1941) that electrically induced convulsions raise the blood sugar in normal

(N) rats, cause hypoglycemia in adrenal demedullated (A) rats, and produce no change in adrenal demedullated-vagotomized (AV) animals. These observations have now been confirmed and extended with regard to the effects of repeated electrical shocks applied 3–12 times, usually on alternate days.

All animals were fasted for 16 hours prior to the test shock, to obtain control sugar values. After repeated shocks, these control values became higher in the N and V groups, and the hyperglycemic response to the test shock became greater in extent and duration. The evidence is interpreted as indicating an increased reactivity of the sympathetico-adrenal (S-A) system.

In the A group, where the action of the S-A system is eliminated, repeated shocks abolished the usual hypoglycemic response to the test shock, but did not change the control value. This indicates a decreased reactivity of the vago-insulin (V-I) system.

In the AV group, where both the S-A and V-I centers were blocked, there was no change after repeated shocks.

It is noteworthy that a greater general excitability was seen in those rats where either the sympathetic centers were excited or the parasympathetic centers were depressed.

An assay method for corticotrophin. E. B. Astwood and R. Tyslowitz (by invitation). *Depts. of Pharmacology and Medicine, Harvard Medical School, and the Medical Clinic of the Peter Bent Brigham Hospital, Boston.* Male rats were hypophysectomized at an average age of 28 days and average weight of 55 grams and injected during the subsequent 5 days with 8 equal doses of pituitary preparations. The adrenal weights 120 hours after operation of 200 animals treated with increasing doses of a single preparation of sheep pituitary powder were fitted to a logistic curve in the logarithm of the dose. The resulting curve can be expressed by the relation:

$$y = \frac{7.6\, x^{2.9}}{x^{2.9} + 127.550} + 5.91,$$

wherein y is the adrenal weight in milligrams per square decimeter of body surface area and x the total dose of the standard powder in milligrams. This standard assay curve exhibits a point of inflexion near the middle of its steepest portion which corresponds closely to the normal adrenal weight of unoperated, untreated animals. Consequently, a unit of corticotrophic activity is tentatively defined as the total amount of hormone required, under the above conditions, to maintain the weight of the adrenals of hypophysectomized rats at a normal level for 5 days. This unit is contained in approximately 50 milligrams of commercial sheep pituitary powder.

A similar dose-response relationship, but with a somewhat higher upper limiting value, has been found to hold for purified extracts of sheep pituitary glands and for the acetone-dried powders of human and pig pituitaries. However, horse and cattle pituitaries differ qualitatively and elicit a strictly limited adrenal response. The potency of the hypophyses of the species studied decreases in the order pig, man, sheep, horse, and cattle.

The localized effect on the spinal cord of intramuscularly injected tetanus toxin. G. H. Acheson and O. D. Ratnoff (introduced by A. Rosenblueth). *Dept. of Physiology, Harvard Medical School, Boston, Mass.* The theory that tetanus toxin may be carried to the spinal cord along peripheral nerve trunks was attacked by Abel (Science **79:** 122, 1934, and elsewhere). In the present study local tetanus limited to one hind limb was produced in cats by the injection of small amounts of toxin into the calf muscles of that limb. Reflex discharges from the spinal cord were recorded electrographically and myographically from the motor nerve or the calf muscles. While the responses of the spinal cord on the unaffected side remain unchanged, those on the affected side develop characteristic changes parallel with the peripheral signs of local tetanus. This localized effect on the cord is not dependent on changes in the peripheral neuromuscular elements or the dorsal root ganglia. It develops even though the muscular signs of local tetanus are prevented by extensive denervation of the leg muscles 18 to 20 hours after injection of the toxin. Hence tetanus toxin, injected into a muscle, affects selectively the regions of the spinal cord from which the innervation of that muscle arises. Furthermore, the spinal changes may be prevented if the leg is denervated before the injection of toxin. It may be concluded that the toxin ascends the nerve trunk to the spinal cord.

Creatinuria following the ingestion of caffeine. George Bachmann, John Haldi, Charles Ensor (by invitation) and Winfrey Wynn (by invitation). *Dept. of Physiology, Emory Univ., Ga.* (Read by title.) In a study of the effect of caffeine on the respiratory exchange in man, creatine was found in the urine of three adult male subjects in 30 out of 35 experiments in which pure caffeine alkaloid was taken in solution in amounts varying from 1.8 to 6 mgm. per kilo body weight. In four experiments the excretion varied from 0.7 to 1 mgm. per hour and in the remainder from 1.1 to 3.3 mgm. per hour. No definite relationship was observed between the amount of caffeine ingested and the rate of creatine excretion. Analysis was made of the urine collected one hour and forty-five minutes after taking the caffeine.

With a smaller amount of caffeine, namely, 1 mgm. per kilo, creatine appeared in the urine in

only 5 out of 24 experiments. The rate of excretion varied from 0.9 to 1.4 mgm. per hour. In two of these experiments creatine was excreted in the post-absorptive period at practically the same rate as in the post-ingestion period. There was no creatinuria in eighteen control experiments in which water alone was taken.

The data of these experiments were grouped with corresponding data in other experiments in which creatinuria occurred (Am. J. Physiol., 115: 364, 1936) to determine whether there was any relationship between the respiratory exchange and creatine excretion. There was found, however, no correlation between the amount of creatine excreted and the total oxygen consumption, total carbon dioxide eliminated, or the percentage increase of oxygen or of carbon dioxide above the basal level.

The effect of dust inhalation on susceptibility to pneumonia. ANNA M. BAETJER and FREDERICK J. VINTINNER (by invitation). *Dept. of Physiological Hygiene, Johns Hopkins School of Hygiene and Public Health, Baltimore, Md.* Rats were exposed 8 hours daily to high concentrations of fine dusts (quartz, feldspar, or portland cement) for various periods from one to 157 days before intrabronchial inoculation with type I pneumococcus in mucin (Nungester method). In some experiments dust exposure was continued after inoculation. In every experiment exposure to silica, feldspar or a portland cement of Belgian origin increased the resistance of rats to the pneumococcus organisms given in mucin, as evidenced by a lower mortality and a lower incidence of lung consolidation in the dusted groups as compared with control rats kept under similar conditions without dust. The resistance increased with the duration of exposure before inoculation but exposure after inoculation was also somewhat beneficial. Rats with extensive silicosis showed a marked resistance to pneumonia. An American portland cement sample, tested for relatively short periods, did not alter the resistance, indicating that only certain types of dust may be effective.

The increased resistance to the pneumococcus organisms in mucin is probably due to the increase in the number of phagocytic cells in the lung tissue resulting from the presence of the dust, and secondly, to the adsorption of mucin by the dust thus allowing the defense mechanisms to attack the organisms, since mucin facilitates the survival of organisms in the body.

Fructose metabolism in the fasting dog. S. B. BARKER, J. A. DYE and WILLIAM H. CHAMBERS. *Dept. of Physiology, Cornell Univ. Medical College and New York Hospital, New York City.* Previous investigators have disagreed concerning the effect of fasting on the metabolism of administered fructose. Therefore, the changes in blood lactate, glucose and fructose, as well as in respiratory metabolism following the ingestion of fructose have been determined in dogs fed standard diets and after three weeks of fasting.

In the post-absorptive state during a standard diet regimen, 50 grams of fructose caused during the first hour an average rise in blood fructose to 35 mgm. per cent and in blood lactate to 27 mgm. per cent, with a gradual fall almost to basal level by the fourth hour. Blood glucose did not change or fell slightly. After the fast the changes in blood fructose and lactate were only slightly greater (5 to 10 mgm. per cent) but were more prolonged. Blood glucose increased about 30 mgm. per cent.

Respiratory quotients in the standard diet control experiments averaged 0.78 for the basal periods and 0.91 for the second, third and fourth hours after fructose. Following the fast the basal average was 0.73 and after fructose 0.85. The respiratory quotients are reduced only 0.02 or less when a correction for the change in blood lactate is applied to 50 per cent of the body weight. Thus, in contrast to glucose, the oxidation of fructose as measured by the rise in respiratory quotient is not markedly impaired by fasting. These results are in agreement with the concept that fructose may be oxidized without transformation into glucose.

Basal metabolism in acne vulgaris. BRODA BARNES. *Univ. of Denver.* Some of the earlier investigators reported that the basal metabolism in acne vulgaris was normal and hence the thyroid was not involved. No significant data were presented however. Recently some of the dermatologists have felt that this skin disorder was improved when, in addition to the usual therapeutic measures, small doses of thyroid were administered. In view of these conflicting reports it seemed necessary to reinvestigate the problem.

Basal metabolism tests were run on 85 college students which included both sexes. Three of the group were found to be plus or minus zero while twelve were found to be above zero on the first test. Some of these however would fall below zero if a second test were run. Eighty per cent of the total group were below zero on the first test although some were within the normal range. There was some correlation between the severity of the acne and the metabolism. Readings below minus 20 were common in the severe cases. In some cases there were other symptoms of hypothyroidism although the basal was not outside the range usually considered normal.

All of the cases are under thyroid therapy at the present but it is too early to evaluate the results. It seems significant that some of the severe cases are improving which had been resistant to other

forms of therapy. The results suggest that hypothyroidism may be one factor in the etiology of acne vulgaris. Low tissue resistance and poor peripheral circulation might be contributing factors.

Electrical oscillations in a living membrane produced by vibrating tuning forks. T. CUNLIFFE BARNES and T. L. JAHN (introduced by R. Beutner). *Depts. of Pharmacology, Hahnemann Medical College, Philadelphia, and of Zoölogy, Univ. of Iowa, Iowa City.* Frog skin was mounted in Ringer's solution in holder previously described (Barnes, Am. J. Physiol. **130:** 557, 1940). Resting potential was registered by a Micromax recording potentiometer and rapid fluctuations by a cathode ray oscillograph. Piezoelectric monophasic waves were set up by tapping the skin with an insulated rod and by allowing a vibrating tuning fork to come in contact with the skin or solution. Vibrations of 1, 5, 100, 256 and 448 per sec. were followed accurately by the skin potential (microphonic effect). The waves were opposite in sign to the charge of the surface treated. The polarity was reversed by puncturing. Abolition of the resting potential by permanganate or mercury destroyed the piezo-electric effect. The experiments indicate that changes in the tension of living membranes may produce marked electrical effects in the organism. In the ear the cochlear phenomenon of Weaver and Bray may involve piezo-electrical changes in Reissner's membrane as suggested by Rawdon-Smith. The response of muscle spindles and other tension receptors may also include analogous factors.

Possible explanations are available: 1, determinations with a Fenn respitometer indicate that mechanical deformation increases oxygen consumption of frog skin; 2, in models of phase boundary potential agitation of the oil produces fluctuation of the potential; 3, according to Gatty tension increases the ration of anodic (intercellular) to cathodic areas in the skin; 4, tension increases the permeability of the skin which may depolarize.

The neuromyal junction as a peripheral contributor to the regulation of muscular activity. JOHN W. BEAN. *Dept. of Physiology, Univ. of Michigan, Ann Arbor.* (Read by title.) Rapid application of positive intrapulmonic pressure (160 mm. H_2O) diminished the tibialis anticus contractions elicited by regularly repeated stimulation of either the intact or peripheral end of the sectioned peroneal nerve in dogs anesthetized with morphine-urethane, with the vagi intact, cold-blocked or sectioned. This diminution is apparently not due to altered aeration of the lungs, or changes in blood pressure. Preliminary experiments would seem to indicate that the sympathetic supply plays no essential role in the occurrence of this phenomenon. It is suggested that it is induced by some humoral mechanism brought into play by stimulation of proprioceptors in the respiratory muscles by the positive intrapulmonic pressure.

Since the phenomenon does not occur when the tibialis or sartorius muscles are stimulated directly in their nerve free regions it may be concluded that its occurrence during nerve stimulation is dependent upon some influence operative at the neuromyal junction and that the neuromyal mechanism is an important peripheral contributor to the regulation of muscular activity. The not infrequent finding that the initial effects of hemorrhage on the contractions elicited through nerve stimulation and on those elicited by direct muscle stimulation are not immediately parallel; and the pronounced initial increase in strength of contractions elicited by nerve stimulation so frequently observed at death of the animal when the tissues must be turning acid, lend further support to this view. The neuromyal junction apparently provides a potentiating mechanism under these conditions not present in muscle itself to the same degree.

On the specificity of renin and some related phenomena. JOHN W. BEAN. *Inst. de Fisiologia, Univ. of Buenos Aires, Argentina.* The problem of specificity of renin was investigated in preparations from the toad, snake, shark, chicken, duck, dog, sheep, cow, and pig by either the in-vivo (direct injection of renin) or the in-vitro (preparation of hypertensin by incubation, Braun-Menendez et al. (Rev. Soc. Argent. biol. **15:** 161, 1939)) method or by both. Mammalian renin failed to give any pressor response in forms below the mammal. Chicken renin reacted positively only with chicken blood. The poikilotherm preparations failed to show any positive reaction either with the higher forms or among themselves. The injection of toad renin into the toad with body temperature at 18° or 27° failed to elicit any pressor response. Incubation (37°C) of toad renin with toad plasma failed to produce any hypertensin. It would appear, therefore, that renin is absent in poikilotherms. The pressor response in the chicken to injection of hypertensin is essentially the same as in the dog: in poikilotherms (toad and snake) the response is slower in onset, more persistent and frequently exhibits a double peak curve. The persistence is in all probability due to a slower destruction of hypertensin at the lower body temperatures since hypertensinase, which was demonstrated to be present in toad blood, is practically inactive at 18° and optimally active at about 40°. The temperature-activity curves for chicken and mammalian hypertensinase closely approximate that for the toad. Blood pressure re-

cordings in the lower forms show tachyphylaxis to hypertensin especially with the larger doses.

The efficacy of O_2 and O_2-CO_2 mixtures for resuscitation of the newborn. R. F. BECKER and W. F. WINDLE. *Northwestern Univ. Medical School, Chicago, Ill.* Asphyxia neonatorum was induced experimentally in guinea pigs at term. The mother's abdomen was opened under local anesthesia, and one fetus was delivered for a control. Other fetuses were asphyxiated in utero by clamping the uterine vessels or the umbilical cord. When the intrauterine respiratory movements, induced by the anoxia, had ceased (after 5 to 7 min.), the fetuses were delivered. Resuscitation was begun 10 to 15 minutes later when the heart beat had become too weak to palpate. A hyperdermic needle attached to a rubber gas bag then was inserted into the exposed trachea, and the lungs were inflated rhythmically by positive pressure. The heart improved in a few minutes. Breathing began 20 to 60 minutes later.

With oxygen alone, respiration did not begin until the central nervous system had recovered sufficiently to allow afferent vagal and spinal impulses to affect the respiratory center. Each inflation of the lung with oxygen at this time produced a reflex gasp. Pure nitrogen also produced typical Hering-Breuer responses. However, acapnia persisted when pure oxygen was used and artificial respiration had to be continued for some time before automatic firing of the center began.

With 10 per cent carbon dioxide in oxygen, spontaneous responses of the respiratory center were obtained sooner than with oxygen alone. They appeared before afferent vagal and spinal impulses could induce breathing. Ten per cent carbon dioxide in oxygen is preferable to pure oxygen for resuscitation where narcosis and general anesthesia have not been employed.

The protective action of vitamin C against experimental liver damage. KARL H. BEYER (introduced by Walter J. Meek). *Dept. of Physiology, Univ. of Wisconsin Medical School, Madison.* Preliminary experiments using 3 groups of 10 to 20 guinea pigs showed that there was no significant difference with respect to lipoid content and histology between the livers of normal guinea pigs, those on a scorbutogenic diet killed at the time of cessation of weight gain (about 16 to 18 days) and animals on the same diet but injected with 30 mgm. ascorbic acid for 18 days.

The purpose of this study has been to determine whether ascorbic acid given to one of two series of guinea pigs placed on a scorbutogenic diet would protect that group against liver damage. Two groups of 18 animals each were placed on a vitamin C deficient diet. One group was injected subcutaneously with 30 mgm. ascorbic per day. At the time the vitamin C deficient pigs ceased to gain weight all the animals in both groups were injected with 25 mgm. hydrazine sulfate per Kg. on two alternate days. Forty eight hours after the second injection all the pigs were sacrificed and their livers examined grossly, microscopically and by analysis especially for lipoid content. There was 50.3 per cent more lipoid material in the livers of vitamin C deficient animals (23.3 per cent of dried liver weight, average) than those on an adequate vitamin C administration (15.5 per cent of dried liver weight, average).

The livers of the deficient guinea pigs showed marked gross and microscopic evidence of extensive fatty degeneration and necrosis. The animals that received ascorbic acid showed only mild manifestations of the early stages of the pathogenesis of fatty necrosis.

From these data ascorbic acid seems a definite factor in the maintenance of liver function and structure.

The normal pneumocardiogram. H. A. BLAIR, A. M. WEDD (by invitation) and H. M. HARDWICKE (by invitation). *Depts. of Physiology and Medicine, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.* By means of a pneumograph and Frank capsule pneumocardiograms of the chest and abdomen, taken during suspended breathing, were recorded photographically and were related through the electrocardiogram and heart sounds to venous and arterial pulses and to the mechanical events of the cardiac cycle. Collapse of the chest began about 0.13 sec. after the beginning of the Q wave, indicating excess of arterial outflow over venous return during systole. At varying times during the Q-T interval maximum collapse occurred, after which arterial output was exceeded by venous return and the thorax expanded. Closure of the aortic valve was seldom discernible but the beginning and the end of ventricular relaxation were usually plainly marked. Relaxation began about 0.02 sec. after the onset of the second heart sound. It was evidenced by rapid expansion of the thorax and was usually followed by a slight rebound. It is believed that ventricular relaxation causes the rising phase of the venous "v" wave and, likewise, the dicrotic notch of the arterial pulse. The pneumogram usually indicated the onset of isometric contraction of the ventricle; this frequently occurred as early as 0.01 sec. after the beginning of the Q wave. Ventricular relaxation followed the peak of the T wave at varying intervals in different subjects. During ventricular ejection abdominal collapse also occurred, beginning somewhat later than that of the chest, which indicated that the abdomen lost more venous blood to the chest during systole than it gained from the periphery.

Observations pertaining to the localization of nerve fiber pathways subserving heat regulating

functions in the midbrain and pons. J. R. BLAIR (by invitation) and A. D. KELLER. *Dept. of Physiology and Pharmacology, Univ. of Alabama, University.* Procedures for the quantitative calibration of heat regulating ability of normal dogs have been described (J. R. Blair and A. D. Keller, Am. J. Physiol. **133:** 229, 1941). These procedures were utilized in detecting the presence or absence of defects in regulating ability of dogs having their brainstems cut crosswise, with varying degrees of completeness, at the cephalic midbrain and middle pons levels.

Hemisection of the brainstem at these levels produces no detectable defect in either the heat maintenance or heat loss mechanisms.

A dog's heat maintenance ability can be entirely eliminated by a transverse cut which involves the medial portions of the right and left halves of the brainstem (medial quarter segments) at the cephalic level of the midbrain. A lesser medial cut spares this mechanism in part or entirely according to the width of the section. A transection except for a millimeter or two of tissue immediately adjacent to the midline leaves the animal with considerable heat maintenance powers. These observations verify Isenschmid and Schnitzer's conclusions (rabbit) that the heat maintenance fibers in coursing from the hypothalamus to the midbrain have a diffuse medial distribution.

At the pons level the heat loss fibers are eliminated by transverse sections involving only the lateral portions of the brainstem. A transection of the brainstem except for the sparing of one extreme lateral segment does not impair heat loss powers nor eliminate heat maintenance powers. A deficit in heat maintenance powers has, however, been encountered in such preparations. These results verify on a better quantitative basis previous observations by one of us (A. D. K.).

The effect of ultraviolet radiation on body weight of mice. H. F. BLUM, H. G. GRADY (by invitation) and J. S. KIRBY-SMITH (by invitation). *National Cancer Inst., National Inst. of Health, U. S. Public Health Service, Bethesda, Md.* Conflicting statements have been made regarding the effect of light and of ultraviolet radiation on the growth of animals. We have had opportunity to study the effect of mercury arc radiation on the body weight of male albino mice (strain A) incidental to studies of the induction of cancer. Such radiation, which includes the sunburn-producing wavelengths shorter than 3200A, was applied at regular intervals in various doses and intensities. Decreased rate of gain in body weight resulted. At comparable stages of the experiments, the average weight and the logarithm of the dose are linearly related down to rather low doses below which no effect on weight is observable. Intensity of radiation *per se* is not a factor in this inhibition. Degree of skin damage parallels inhibition of weight gain. There are no changes in internal organs suggestive of major systemic effects. The slower gain in body weight is probably accounted for by decreased food intake resulting from superficial damage.

Hyperventilation as a retarding factor in oxygen poisoning. DAVID F. BOHR (by invitation) and JOHN W. BEAN. *Dept. of Physiology, Univ. of Michigan.* (Read by title.) The increased susceptibility of rats to oxygen poisoning induced by the addition of small amounts of CO_2 has been interpreted recently (Massart, Compt. rend. Soc. Biol. **117:** 265, 1934) as due to an increased uptake of O_2 consequent upon a hypercapnic hyperpnea rather than to the disturbed CO_2 transport. In an experimental evaluation of hyperpnea as a possible contributing factor in O_2 poisoning the effects of artificial hyperventilation on adult meal tracheotomized rats anesthetized with urethane and exposed to O_2 at 6 atmospheres were studied. The respirator employed provided means of accurately regulating and recording minute volume of normal and artificial ventilation. Under normal ventilation terminal apnea appeared in 77 minutes of exposure to O_2 at 6 atmospheres. Rats decerebrated under transient ether anesthesia appeared to be similarly affected. On the other hand animals subjected to 2 minutes of artificial hyperventilation and 3 minutes of natural ventilation alternately throughout the exposure, became terminally apneic only after 129 minutes. It may be concluded therefore that hyperventilation *postpones* the toxic action of O_2 rather than hastens its onset in spite of a possibly more rapid O_2 saturation of the tissues. This postponement is probably due to an improved CO_2 diffusion gradient between plasma and alveolar gas. The finding that the terminal apnea appeared as early as 33 minutes of exposure when the rats rebreathed the accumulating CO_2 without artificial ventilation, further supports the disturbed CO_2 transport interpretation of O_2 poisoning.

Rôle of the liver in experimental uremia. ADRIAN BORDUAS (by invitation), CHRISTIANE DOSNE (by invitation) and HANS SELYE. *Dept. of Anatomy, McGill Univ., Montreal, Canada.* In order to study the rôle of the liver in uremia, experiments were performed on male albino rats two groups being bilaterally nephrectomized, one of them having simultaneously undergone removal of 70 per cent of the hepatic tissue (method of Waelsch and Selye, 1931). A third group was merely partially hepatectomized according to the same technic. We found that while the nephrectomized rat survives 48–72 hours, nephrectomized and partially hepatectomized animals die within 19 hours after the operation. With very few exceptions all animals survive this type of partial

hepatectomy indefinitely thanks to the rapid regeneration of the liver tissue. The slope of the blood N.P.N. curve following nephrectomy is not significantly changed by simultaneous partial hepatectomy. The blood N.P.N. at the time of death is consequently much lower in the partially hepatectomized and nephrectomized, than in the merely nephrectomized rat. The blood sugar concentration shows a transitory decline after partial hepatectomy, but its degree is not influenced by simultaneous nephrectomy. Prevention of this hypoglycemia by parenteral glucose administration temporarily improves the general condition of the partially hepatectomized and nephrectomized animals, but maintenance of a normal blood sugar level does not prolong their survival significantly. Our experiments indicate that hepatic detoxification probably plays an important part in the defence of the organism against toxic metabolites usually dealt with by renal excretion.

The effect of histamine antagonists on gastric secretion. JOSEPH E. BOURQUE (by invitation) and EARL R. LOEW. *Wayne Univ. College of Medicine, Detroit.* Several investigators have indicated that thymoxyethyldiethylamine (929F) and N-phenyl - N - ethyl - N′ - diethylethylene diamine (1571F) antagonize the effect of histamine on smooth muscle. These reports suggested the use of histamine antagonists in a study concerning the relation of histamine to gastric secretion.

Dogs with vagotomized gastric pouches (Heidenhain) were given non-toxic doses of 929F or 1571F (10 mgm. per kgm.), subcutaneously, 20 to 30 minutes before the injection of the gastric secretory stimulants employed.

Fifteen experiments on 3 dogs treated with 1571F yielded data which indicated that the volume, free acid, and total acid of the gastric juice following histamine injections were reduced 21, 47 and 35 per cent, respectively, from control values obtained by injecting histamine alone earlier the same day. This inhibitory effect was probably not due to a specific antagonism to histamine for similar experiments revealed that 1571F suppressed the gastric secretory stimulating action of pilocarpine to the same degree.

In 15 trials, 929F failed to appreciably affect the volume or acidity of gastric juice secreted following pilocarpine administration. It was previously found that this drug, instead of antagonizing histamine, apparently potentiates the effect of histamine on the gastric glands.

Although 1571F inhibited gastric secretion, there was no indication that either 1571F or 929F, with the doses employed, specifically antagonized the effect of histamine on the gastric glands.

Circulatory effects of brief passive inflation and deflation of the lungs. T. E. BOYD and MARY C. PATRAS (by invitation). *Dept. of Physiology and Pharmacology, Loyola Univ. School of Medicine, Chicago.* In dogs under barbital anesthesia, the lungs were passively inflated or deflated by brief application of positive or of subatmospheric pressures through the trachea. Resulting changes of ventricular volume and output were studied by the plethysmographic technique described earlier (Am. J. Physiol. **134:** 74, 1941). The chest was closed and the ventricles left under intrathoracic pressure.

Starting with the lungs in the position of normal expiration, inflation by a positive pressure of 12 cm. H_2O reduces ventricular volume and stroke output, the latter by one-fourth to one-half or more. Venous pressure at the entrance to the right auricle, measured against intrathoracic pressure, is reduced. The carotid pulse is weakened, but this effect does not appear until two or three beats after the first reduction of combined stroke output. Passive inflation thus appears primarily to impair filling of the right ventricle.

Suction of air from the lungs, by an intratracheal pressure 8 or 10 cm. H_2O below atmospheric, augments the effective venous pressure (right side), diastolic volume and stroke output. In some experiments we have found these effects to continue undiminished throughout a period of deflation lasting over 20 or more cardiac cycles; in others they have been maintained only for the first few beats, effective venous pressure thereafter returning gradually to the normal expiratory level.

Local potentials and tonus changes in cardiac muscle and their relation to the initiation of impulses. EMIL BOZLER. *Dept. of Physiology, Ohio State Univ., Columbus.* If a monophasic action potential is recorded from the pace-maker in a strip of cardiac muscle, each spontaneous impulse is preceded by a period of slowly rising negativity. Weak potential waves continue after the beat has been stopped by high concentrations of acetylcholine. In injured preparations which show Luciani periods each period of activity is preceded by weak, non-conducted potential oscillations of gradually increasing magnitude. The impulses of each period are discharged at a negative crest of the oscillations. Under the influence of an excess of Ca ions and other conditions rhythmic after potentials occur. These potentials are purely local and, if they reach a sufficient magnitude, they initiate a discharge of impulses in a previously quiescent preparation. In strips and in the whole ventricle of the turtle it can be shown that the after potentials are accompanied by small changes in tension, and, therefore, are due to changes in the activity of the muscle. The results suggest that spontaneous impulses in cardiac muscle are initiated by local changes of the activity. The presence of rhythmic after potentials,

only found in injured cardiac muscle, perhaps explains some of the disturbances of rhymicity often ascribed to a reentry of impulses.

Examples of the acid-neurohumoral mechanism of nervous integration. CHARLES R. BRASSFIELD and ROBERT GESELL. *Dept. of Physiology, Univ. of Michigan, Ann Arbor.* An acid mechanism of controlling the duration of survival of physiologically deposited acetylcholine in the respiratory center could obviously be an effective means of controlling respiration. This paper however inquires into the extension of this principle into other reflex arcs. Stimulation of the peripheral end of the vagus nerve of the dog has been found to produce greater slowing of the heart during hypercapnia than eupnea. Greater slowing also occurred on central stimulation with the opposite vagus intact. The isolated turtle's heart previously acidulated in carbonated Ringer's solution was more powerfully inhibited when immersed in a weak solution of acetylcholine in non-carbonated Ringer's solution than the unacidulated heart. The previously acidulated intestine of the turtle and rabbit, and rectus abdominus of the frog were also found more reactive to acetylcholine than the untreated preparations. It has been thoroughly demonstrated that the response of the submaxillary gland to pilocarpine is greatly augmented by administration of a gaseous mixture of CO_2. Evidence is now at hand showing that the response of the gland to acetylcholine is also frequently enhanced by similar procedure. Ammonium chloride which consistently produces an acid saliva invariably augmented the flow activated by pilocarpine, acetylcholine, or chorda-tympani stimulation.

The secretion of renin by the normal kidney. EDUARDO BRAUN-MENENDEZ (introduced by Hallowell Davis). *Univ. of Buenos Aires Medical School, Argentina.* It is regretted that an abstract of this paper could not be secured in time for publication. *Ed.*

Cortico-adrenal extract influence on carbohydrate levels in thyroidectomized rats. S. W. BRITTON and C. R. FRENCH (by invitation). *Physiological Laby., Univ. of Virginia Medical School, University.* (Read by title.) Several series of thyroidectomized rats have been studied under different experimental conditions. All animals were followed for a week after operation, and then utilized for tissue samples while still in good condition. The extract used was prepared in this laboratory.

Decreases in blood sugar and muscle glycogen in untreated rats after thyroid excision were moderate in degree; severe reductions were observed, however, in liver and heart glycogen levels.

In similar thyroidectomized animals which were given cortico-adrenal extract (5 cc. twice daily) after operation, all carbohydrate levels were found to be much higher, and indeed within normal limits. Notably, it was observed that liver glycogen values after extract treatment averaged 100 per cent and cardiac glycogen 150 per cent above those in control rats.

Similar changes in carbohydrate levels, and similar effects of adrenal extract, were also noted in thyroidectomized rats which were partially adrenalectomized (left adrenal gland removed).

No significant differences were observed in serum electrolyte levels (Na, K, Cl) in thyroidectomized rats with or without adrenal extract treatment.

The results further emphasize the specific action of cortico-adrenal extracts on carbohydrate levels in the organism under critical conditions. They possibly have a bearing also on the alleged thyroid influence on hepatic gluconeogenesis.

On a condition simulating adrenal insufficiency in post-pituitary injected dogs. S. W. BRITTON and R. F. KLINE (by invitation). *Physiological Laby. Univ. of Virginia Medical School, University.* (Read by title.) Studies have been carried out on normal dogs maintained in metabolism cages over periods of several weeks. Water intake, urine output, and urine and serum sodium and chloride determinations were made daily before and after treatment. Rather large doses of post-pituitary extract (0.5 to 1 cc. twice daily per 8 kgm. dog) were administered for periods of one to two weeks.

The water intake and urinary output levels varied considerably from day to day, and extract injections did not appear to influence these values greatly. There were apparent, however, significant changes in urinary and serum electrolyte values after treatment. In all cases, urine sodium and chloride concentrations increased notably during post-pituitary extract administration, while the electrolyte levels in blood serum were concurrently reduced.

These results are in agreement with the hypothesis suggested in earlier papers from this laboratory. About two years ago we showed that corticoadrenal extract and desoxycorticosterone (kindly supplied by Dr. Ernst Oppenheimer of Ciba Products) given in excess may produce a condition much like that of diabetes insipidus. The present work indicates that post-pituitary extract given in large amount over adequate time may force a condition similar to that of adrenal insufficiency.

Further observations on experimental hypothalamic obesity. J. R. BROBECK (by invitation), JAY TEPPERMAN (by invitation) and C. N. H. LONG. *Dept. of Physiological Chemistry, Yale Univ. School of Medicine, New Haven, Conn.* Further study of the obesity produced in rats by hypothalamic lesions has been carried out along 3 lines.

1. Preliminary observations suggested that when they were pair-fed with littermate controls, operated animals gained weight more rapidly than the controls. More carefully controlled experiments, however, have indicated that the rate of gain may be the same in the two types of animals. Of 7 pairs of rats pair-fed a diet of which the dry weight could be calculated daily, 4 of the operated animals obviously gained weight only as rapidly as their controls. In 2 other cases the results were equivocal, but one operated rat gained at a definitely more rapid rate than the control.

2. Experiments with diets composed of mixtures of fresh ground beefsteak or pig liver, corn meal, lard, casein, codliver oil, Mendel salt mixture and water have shown that the operated rats attain considerably heavier body weights on such a diet than on a commercial diet such as "calf meal". Four rats at liberty to choose the separate components of the former diet, postoperatively doubled their total caloric intake but showed no special choice of any article of the diet.

3. As an incidental autopsy finding, the kidneys of one fat rat showed marked pathological changes in the glomeruli and tubules. Subsequent examination of the urine of 11 operated and 9 normal rats disclosed, in 9 of the operated animals, significant albuminuria frequently associated with casts. [*This work was assisted by a grant from the John and Mary R. Markle Foundation.*]

Experimental production of obesity in the monkey (Macaca mulatta). CHANDLER MCC. BROOKS, E. F. LAMBERT (by invitation) and PHILIP BARD. *Dept. of Physiology, Johns Hopkins Medical School, Baltimore, Md.* Obesity developed in six of twelve monkeys after lesions were made in the suprachiasmatic areas and in the tuberal region of the hypothalamus. Eight animals were mature and their body weights (4 to 6 kgms.) were relatively stable before operation. Judging by body and limb dimensions, two of the four immature monkeys failed to grow following the operation. One of these became obese.

Three weeks after operation weight gain became apparent in the six animals. They gained at a rate varying between 50 and 150 grams per week until their body weights more than doubled. This period of rapid increment lasted eight to ten months. The weights then remained relatively constant. Excess fat was distributed generally but the heaviest deposits were subcutaneous, chiefly abdominal, and in the omentum and mesenteries.

With normal feeding none of the twelve animals showed acetonuria, glycosuria, insulin sensitivity or abnormality in sugar tolerance. The total daily caloric intake of the obese monkeys was much greater than that of non-obese animals but their caloric intake per kilogram was somewhat less. When deprived of food obese animals lost weight slightly less rapidly than did non-obese monkeys. Acetone bodies appeared in the urine of all animals on the third day of starvation.

The obesity was not dependent on certain other deficiencies. It occurred in association with and also in the absence of hypogonadism, dwarfism and diabetes insipidus. Diabetes insipidus, failure of growth and hypogonadism were produced in other monkeys by lesions which did not cause obesity. [*This work was aided by a grant (to Dr. Philip Bard) from the Committee for Research in Problems of Sex, National Research Council.*]

Decrease in insulin tolerance following hypophysectomy largely due to removal of stalk tissue. J. M. BRUHN, A. D. KELLER, W. E. LAWRENCE (by invitation) and C. F. BARTON (by invitation). *Dept. of Physiology and Pharmacology, Univ. of Alabama, University. Procedures.* Dogs were prepared by cutting through the hypophysis with scissors at various distances from its attachment with the hypothalamus. The glandular tissue distal to the section was removed; that proximal to the section was left with its normal hypothalamic attachments. The animals were calibrated by determining 1, the largest subcutaneous dose of insulin tolerated with spontaneous recovery, and 2, the smallest dose precipitating a major convulsion necessitating sugar administration for relief of the hypoglycemic crisis. Calibration studies were begun not earlier than two months after hypophysectomy. Operative procedures were verified histologically after the experiments were terminated. *Removals and results.* A *near-total ordinary hypophysectomy* (more than 90 per cent of the distal pars anterior and posterior lobe removed) mildly reduces insulin tolerance (3–5 times), produces partial or complete sexual regression, partial coat change, stoppage of growth, mild adrenal atrophy and a tendency to increase fat stores on a diet normally not inducive to fat storage.

An *ordinary hypophysectomy* (elimination of the entire pars anterior, the posterior lobe, and some of the distal tuberalis) results in a comparatively small (10 times) reduction of insulin tolerance, complete sexual regression, stoppage of growth, maximal coat change, moderate adrenal atrophy, and a definite tendency to increase fat stores.

However, following a *near-total hypophysectomy* (an ordinary hypophysectomy plus removal of varying amounts of the stalk) a *greatly diminished* insulin tolerance (20–80 times depending roughly on the level of stalk section) occurs. Adrenal atrophy is definitely greater and fat deposition more pronounced than following ordinary hypophysectomy.

Evidence against the alleged peptogogic effect of the hormone, secretin. G. R. BUCHER (by invita-

tion) and H. GREENGARD. *Dept. of Physiology and Pharmacology, Northwestern Univ. Medical School, Chicago.* (Read by title.) The report of Pratt (1940) in England, that secretin is a good peptogogic agent renders it necessary to present our evidence, which refutes his conclusions and reaffirms the data recorded in the early history of this hormone. Our evidence is derived from the results of ten experiments performed on 1 Pavlov pouch and 9 total gastric pouch dogs. Gastric secretion in these animals was studied with regard to total volume, acid and pepsin outputs during a 3-hour interval before and again immediately after the intravenous administration of 1 mg. of crystalline secretin. This dose of secretin was known to be active and to be 70 times the threshold dose for the dog.

The data are presented as averages representing 3-hour interval outputs:

	Volume, cc.	Total acid, mgm. HCl	Pepsin Hb, units
Pre-secretin interval...	17.53±2.36	15.44±9.11	1.738±0.460
Post-secretin interval..	19.08±3.82	11.61±5.76	2.233±0.724

Since the difference between the observed averages is, in all three comparisons, less than the respective errors of the means, no stimulation of gastric juice, acid output, or pepsin output occurred after the administration of 1 mg. of crystalline secretin of proven activity. Pure secretin, therefore, has no peptogogic capacity.

Action of insulin on pyruvate formation in depancreatized dogs. ERNEST BUEDING (by invitation), JOSEPH F. FAZEKAS (by invitation) and HAROLD E. HIMWICH. *Depts. of Medicine and Chemistry, New York Univ. Medical College, New York City and the Dept. of Physiology and Pharmacology, Albany Medical College, Union University, Albany.* Following intravenous injection of glucose (2 grams per kgm.) a significant rise in blood pyruvate occurs in normal dogs. A similar injection of glucose into depancreatized dogs does not produce any increase in pyruvate. If insulin is administered to depancreatized dogs either simultaneously with glucose or 90 minutes after, a marked elevation in blood pyruvate is observed; its maximum is reached from one to three hours after insulin. A second glucose injection four hours after insulin administration is followed by a further rise in pyruvate. When the blood sugar is raised to 750–950 mgm. per cent for a period of 3 to 5 hours by continuous infusion of glucose, an increase in blood pyruvate takes place in depancreatized dogs despite the absence of insulin. Under these conditions the blood pyruvate reaches a constant level within 1 or 2 hours. A further increase in blood pyruvate is produced by the injection of insulin after 3 hours of glucose infusion. The rate of disappearance of intravenously injected sodium pyruvate from the blood is the same in normal and in depancreatized dogs.

These results indicate that insulin increases pyruvate formation after the administration of glucose to depancreatized dogs.

The effectiveness of intramesenteric androgen pellets in the rat. M. W. BURRILL (by invitation) and R. R. GREENE. *Dept. of Physiology and Pharmacology, Northwestern Univ. Medical School, Chicago.* (Read by title.) Many investigators, using different experimental methods, have consistently shown that natural estrogens are inactivated by the liver. This has been considered the reason for the decreased effectiveness of these substances when administered orally. Although not as extensive, there is also some evidence that natural androgens are inactivated by the liver. In view of the marked oral activity of methyl testosterone compared to other androgens, it would be expected that this substance might not be inactivated by the liver. Biskind, however, found that it was. The possibility remained that the liver might be relatively less efficient in the inactivation of methyl testosterone than in the case of other androgens such as testosterone. In order to test this possibility pellets of testosterone and methyl testosterone were implanted into the mesentery of the small bowel in castrated male rats. Vascular drainage from this site is exclusively through the portal system. The initial pellet weights were varied in different groups in order to provide for absorption of different daily amounts (0.52–0.08 mgm.). The prostate and seminal vesicle weights of these animals, after 11 or 21 days, were compared with those of control animals castrated the same length of time. In all cases the accessory weights of the animals with pellets were greater than those of the controls, indicating that both substances had passed the liver without being completely inactivated even when the daily absorption from the pellet was only 0.08 mg. These results do not concur with Biskind's finding of complete inactivity when pellets of methyl testosterone and testosterone propionate were placed in the normally situated spleen.

Quantitative studies of direct serum bilirubin. ABRAHAM CANTAROW. *Dept. of Medicine, Jefferson Medical College and Laby. of Biochemistry, Jefferson Hospital.* (Read by title.) Samples of serum from subjects with obstructive jaundice and congenital hemolytic jaundice were mixed with varying quantities of *a*, normal serum, and *b*, concentrated plasma in order to study the influence of varying protein and bilirubin concentrations upon the proportion of "direct" bilirubin. Total and "direct" bilirubin were determined by the method of Malloy and Evelyn.

With increasing protein and constant or increasing bilirubin concentrations (both obstructive

and hemolytic jaundice serum), observed values for direct bilirubin were identical with those calculated from the concentrations in the original samples.

In obstructive jaundice serum, with decreasing bilirubin and increasing protein concentrations (above 10 per cent), observed values for direct bilirubin were lower than the calculated values.

In mixtures of obstructive jaundice serum and normal or hemolytic jaundice serum, with constant protein and decreasing bilirubin concentrations, observed values for direct bilirubin were lower than calculated values.

In mixtures of hemolytic jaundice and normal serum, with constant protein and decreasing bilirubin concentrations, observed values for direct bilirubin were higher than calculated values.

The following conclusions are suggested: *a*, marked increase in protein concentration has no significant influence upon the proportion of direct bilirubin, other factors remaining constant; *b*, the relative increase in the proportion of direct bilirubin in hemolytic jaundice serum and its relative decrease in obstructive jaundice serum under conditions of constant protein and decreasing bilirubin concentrations suggest a fundamental difference in the bilirubin in these two conditions.

Deamination of d-l alanine by kidney slices following administration of growth hormone complex. ATTILIO CANZANELLI and DAVID RAPPORT. *Dept. of Physiology, Tufts College Medical School, Boston.* Young adult rats were given pituitary "Growth Complex" (Ayerst, McKenna, and Harrison), containing some adrenotropic factor, intraperitoneally or subcutaneously in four injections of five Collip rat units per 100 grams over 40 hours. The animals were killed about one hour after the last injection. The QO_2 of kidney slices (Warburg) as well as the changes in amino, ammonia, and urea N of the respiratory medium (modified phosphate buffered Ringer's solution with and without 0.01M d-l alanine) were determined.

In the deamination of alanine by kidney slices from 18 non-injected animals there was a decrease of 7.8 ± 0.52 micrograms amino N per mgm. of dry tissue, an increase of 5.3 ∓ 0.43 micrograms ammonia N, and a decrease of 0.4 ± 0.19 micrograms urea N, so that the combined ammonia and urea N increased 4.90 ± 0.44 micrograms. The QO_2 increased from 21.3 ± 0.56 to 29.3 ± 1.01 or 8.0. In 19 injected animals the results were: a decrease of 7.8 ± 0.30 micrograms amino N, an increase of 4.6 ± 0.24 micrograms ammonia N, a decrease of 0.15 ± 0.11 micrograms urea N, so that the combined ammonia and urea N increased 4.5 ± 0.22 micrograms. The QO_2 increased from 20.7 ± 0.24 to 29.2 ± 0.48 or 8.5.

The results indicate that under the experimental conditions the deamination of alanine was unaffected by previous injection with "Growth Complex." The kidney QO_2 of the injected and non-injected animals was the same and the increased oxygen consumption with alanine was the same in both cases.

Development of cross tolerance to both pentobarbital sodium and delvinal sodium by a single large injection of pentothal sodium in guinea pigs. EMMETT B. CARMICHAEL. *Dept. of Physiological Chemistry, School of Medicine, Univ. of Alabama, University.* Normal guinea pigs were injected intraperitoneally with pentothal sodium to determine the average lethal dose of the drug. The dose varied from 35 mgm. to 60 mgm./kilo.

The animals that survived the above experiments were given a single dose of either 20 mgm. of pentobarbtial sodium/kilo or 40 mgm. of delvinal sodium/kilo. There were 74 animals that received the pentobarbital sodium and 58 animals that received the delvinal sodium. The second injection of the barbiturate was given either 2, 3-4, 23 or 30 days after the initial injection of pentothal sodium. If the doses of pentobarbital sodium and delvinal sodium were given within four days after the dose of pentothal sodium, the length of sleep was either markedly shortened when compared with the results on control animals that received similar doses of pentobarbital sodium and delvinal sodium, or sleep was not produced. The length of hypnosis was reduced also.

There was some cross tolerance 23 to 30 days after the administration of the pentothal sodium as evidenced by the duration of sleep and hypnosis.

Toxicity of pentothal sodium [sodium ethyl (1-methyl-butyl) thiobarbiturate] for guinea pigs. EMMETT B. CARMICHAEL. *Dept. of Physiological Chemistry, School of Medicine, Univ. of Alabama, University.* (Read by title.) The toxicity of pentothal sodium has been tested for both young and adult guinea pigs. The drug was dissolved in distilled water and the fresh solution was injected intraperitoneally. Normal animals were used: (1), 154 young guinea pigs, and (2), 56 adult guinea pigs. Of the latter group, 45 animals had delivered one or more litters. The doses varied by increments of 2.5 mgm. from 35 mgm. to 60 mgm./kilo. The average lethal dose for young guinea pigs was about 50 mgm. to 52.5 mgm./kilo. and the minimum lethal dose was 37.5 mgm./kilo. The number of old animals used is small but the preliminary results indicate that old guinea pigs have slightly more resistance to this drug than the young guinea pigs. The average lethal dose for adult guinea pigs seems to be about 55 mgm./kilo and the minimum lethal dose was 40 mgm./kilo.

Development of tolerance in guinea pigs by re-

peated administration of large doses of paraldehyde. EMMETT B. CARMICHAEL, F. A. KAY (by invitation) and G. W. PHILLIPS (by invitation). *Dept. of Physiological Chemistry, School of Medicine, Univ. of Alabama, University, and Bryce Hospital, Tuscaloosa.* (Read by title.) Paraldehyde was introduced as a therapeutic agent in 1882 by Cervello and the next year Morselli reported the hypnotic and sedative actions of the drug. Toleration to the drug has been more or less a controversal subject since 1889.

Nineteen normal young male guinea pigs, that weighed from 418 grams to 592 grams, were used. The drug was diluted with nine volumes of water and 10 cc. of this dilute solution/kilo were injected intraperitoneally three times per week (Tues., Thurs., and Sat.). This size dose is about twice the therapeutic dose of paraldehyde employed by psychiatrists. The series of injections were continued for four weeks. The average weight of the animals dropped from 499 grams to 469 grams during the experiment.

The periods of time for three stages of the hypnotic state were recorded: 1, the time from the injection until the onset of sleep increased from 92 seconds to 100 seconds; 2, the duration of sleep dropped from 95 minutes to 31 minutes, and 3, the end of hypnosis dropped from 231 minutes to 167 minutes.

Caliber changes of the vessels of the capillary bed. ROBERT CHAMBERS and B. W. ZWEIFACH (by invitation). *Dept. of Biology, Washington Square College, New York Univ.* Vasomotion, a relatively slow and periodic contraction and dilatation, characterizes the arteriolar and precapillary components of the capillary bed. These two muscular components exhibit vasomotion simultaneously and also independently. The film presents various aspects of this functional process in its significance to the normal circulation of the bed.

The vasomotion is recorded in normal and high speed photography. It shows the movement of the cells of the muscle coat and the longitudinal folding of the endothelial coat during the contraction and relaxation phases. It also shows the sphincter-like functioning of the precapillaries at their junctions with the arteriole.

The vasomotion is abolished or altered by mechanical irritation and by the application of histamine, the vessels remaining in their dilated state. With adrenalin the vasomotion is arrested in the constricted phase. The caliber of the non-muscular components, viz., the distal segments of the a-v capillaries, the true capillaries and the small venules, are not affected by these procedures.

Unsaturated lactones related to cardiac glycosides. K. K. CHEN and ROBERT C. ELDERFIELD. *Lilly Research Lab. and Dept. of Chemistry, Columbia Univ.* A series of 31 unsaturated lactones, related to cardiac glycosides, was investigated pharmacologically for the presence of a digitalis-like action. With the exception of nepetalactone, all the compounds were prepared synthetically. Three of them were acetylated strophanthidins, coupled with simple sugars. Briefly, systolic standstill of the frog's ventricle was observed with β, γ-angelica lactone, lactone of 21-hydroxy-$\Delta^{20,21}$-norcholenic acid, methyl coumalate, ethyl coumalate, and the three strophanthidin glycosides. Results of assays by the U.S.P. XI one-hour frog method are summarized in table 1.

TABLE 1

COMPOUND	MEDIAN SYSTOLIC DOSE IN FROGS ± STANDARD ERROR	MEAN (GEOMETRIC) LETHAL DOSE ± STANDARD ERROR
	mgm. per gm.	*mgm. per kgm.*
β,γ-Angelica lactone	3.25 ± 0.15	
Lactone of 21-hydroxy-$\Delta^{20,21}$-norcholenic acid	>0.21	
Methyl coumalate	0.70–1.0	
Ethyl coumalate	0.81 ± 0.06	
Strophanthidin β-tetra-acetyl *d*-glucoside	0.0188 ± 0.003	1.166 ± 0.125
Strophanthidin β-tri-acetyl *d*-xyloside	0.0081 ± 0.001	0.592 ± 0.070
Strophanthidin β-tetra-acetyl *d*-galactoside	0.0113 ± 0.002	1.692 ± 0.168
Strophanthidin	0.0027 ± 0.0005	0.306 ± 0.039

In cats, a typical digitalis-like action was demonstrated with the acetyl strophanthidin glycosides, and their mean lethal doses in groups of 5 cats were determined as shown in table 1. On the other hand, no such effect was present with β,γ-angelica lactone, and methyl and ethyl coumalates.

Effect of various steroids on the vaginal mucosa. ELEANOR CLARKE (by invitation) and HANS SELYE. *Dept. of Anatomy, McGill Univ., Montreal, Canada.* The effect of 45 cyclopentano perhydrophenanthrene derivatives on the vaginal mucosa of immature or spayed adult albino rats was studied. The steroids were administered in peanut oil solution or suspension either subcutaneously for several days or as a single intraperitoneal injection. The former experiments being performed to study the effect of chronic treatment, the latter to establish the action of a sudden flooding of the organism with these steroids. The vaginal changes observed were of 3 types: stratification without cornification, cornification, and mucification. In most cases changes noted by the vaginal smear method were verified by histological examination.

While a detailed discussion of all our observations cannot be given in this brief summary the

most outstanding results are the following. Among the steroids examined all those possessing a side chain at C_{17} of more than two carbon atoms proved devoid of any action on the vagina. All steroids proven to possess any of the known hormonal effects exerted some action on the vaginal epithelium. In some cases this consisted of continuous cornification or stratification, in other cases a transitory cornification was later followed by mucification. The close relationship between these changes is well demonstrated by the fact that in certain instances (e.g., progesterone or D.C.A.) one part of the vagina was cornified while another region was mucified. It appears from our observations that the ability to stimulate the vaginal epithelium is another instance of a pharmacological action common to all known steroid hormones.

1-methyl 4-phenylpiperidine-4-carbonic acid ethyl ester (D-140, demerol): A pharmacologically active substance with atropine-like and morphine-like properties. DAVID R. CLIMENKO. *Research Lab. Winthrop Chemical Co., Inc., Rensselaer, N. Y.* Demerol (D-140) is 1-methyl 4-phenyl-piperidine-4-carbonic acid ethyl ester. There is a structural analogy to the atropine molecule. A similar analogy can be shown to morphine if the older piperidine structure is substituted for the generally accepted phenanthrene configuration.

Demerol possesses both atropine-like and morphine-like properties. It is capable of causing reversible depression of the cardiac vagus of the anesthetized animal.

The drug is a potent spasmolytic agent. This action is in part due to depression of the parasympathetic endings but is primarily the result of a direct (papaverine-like) depression of the muscle fiber. This effect has been observed under experimental conditions on isolated smooth muscle, and in man under clinical conditions.

Therapeutic doses produce a slight sedative, and a marked analgesic action. This has been demonstrated in mice by the Von Killian technique; in rats by the tranquilizing technique of Barlow, by the potentiation of Na Evipal hypnosis, and by the premedication effect in nitrous oxide anesthesia; in cats by the Eddy technique; in dogs and monkeys by observations on responses to stimulation; and in man by the Wolff-Hardy pain-threshold technique and by clinical observation.

Experimental animals cannot be addicted. In man, the analgesic effect persists for 5 to 6 hours. The drug frequently produces marked euphoria which persists for about an hour after administration.

The histamine content of gastric juice. C. F. CODE, G. A. HALLENBECK (by invitation) and R. A. GREGORY (by invitation). *Division of Experimental Medicine, Mayo Foundation, Rochester, Minn.* The histamine content of gastric juice from Pavlov and Heidenhain pouches in dogs has been studied. The histamine in the gastric juice was estimated by a procedure modified after that of Barsoum and Gaddum for the determination of histamine in blood. To obtain histamine concentrations suitable for assay the gastric juice was concentrated during extraction. Also, in order to obtain routinely satisfactory assays the major part of the potassium in the gastric juice had to be eliminated by extraction with absolute alcohol. The minimal concentration of histamine which could be satisfactorily estimated was 2.5 gamma histamine base per liter gastric juice.

Histamine determinations were made on gastric juice produced in response to a meat meal, mecholyl (acetyl-beta-methylcholine chloride) injections and histamine injections. Readily estimable quantities of histamine were present in juice obtained by histamine and mecholyl injections. The highest histamine concentrations occurred in gastric juice produced by mecholyl stimulation. The histamine content of gastric juice secreted during a meat meal was often low and at times was below the minimal quantity which could be detected. When the histamine content of the juice from Pavlov pouches was followed at half hour intervals during the response to a meal, the highest values were always obtained during the first half hour, when as a rule, readily estimable quantities were present. Following this early peak the concentration declined rapidly, usually reaching a plateau level somewhat above the minimal estimable concentration but at times, sinking even below that value.

The effect of ionic environment upon the longitudinal impedance of the squid giant axon. KENNETH S. COLE and GEORGE MARMONT. *Dept. of Physiology, College of Physicians and Surgeons, Columbia Univ., New York.* The alternating current impedance was measured between two large fluid electrodes longitudinally disposed along the squid giant axon and over the frequency range of 10 cycles to 150 kilocycles, after the method used by Cole and Baker (J. Gen. Physiol. **24**: 771, 1941). This method was modified—to obtain an increased survival time of eight hours or longer and more consistent data—by maintaining the interelectrode region in moist air and by leaving both electrode regions unkilled. The impedance characteristics were relatively constant above 1 kilocycle but below this frequency they were highly dependent upon the preparation of the axon and the ionic environment.

Fresh, well prepared axons treated with normal sea water had a high membrane resistance and an unpredicted capacitative reactance below 100 cycles. With increasing calcium the resistance

and this reactance were increased. With decreasing calcium the resistance was decreased and the low frequency reactance became inductive. In the absence of calcium the resistance was 20 per cent of normal and this reactance was highly inductive. Increasing potassium also decreased the membrane resistance and produced an inductive reactance at low frequencies until the potassium concentration reached 8 or 10 times normal when the reactance became capacitative at all frequencies.

These results indicate that the physiological properties of the axon are closely related to its electrical structure and show that this structure is more complicated than previously assumed.

Rôle of a renal humoral mechanism in hemorrhage. DEAN A. COLLINS and ANGIE S. HAMILTON (by invitation). *Dept. of Physiology, Temple Univ., School of Medicine, Philadelphia.* That a renal humoral mechanism is a homeostatic factor in hemorrhage was shown by Hamilton and Collins (Am. J. Med. Sci. **202**: 914, 1941). Sapirstein et al. (Proc. Soc. Exper. Biol. and Med. **48**: 505, 1941) found in blood after hemorrhage a substance acting like renin on ileum, but did not establish a renal origin. These studies on dogs anesthetized with chloralose amplify our previous findings.

Blood (usually 42 cc.) was tested for pressor activity in a nephrectomized dog by injection accompanied by synchronous blood loss. Bloods obtained before hemorrhage caused no significant elevation of blood pressure. In 8 intact dogs samples taken about one hour following hemorrhage (34–45 cc. per kgm. of body weight) invariably gave rises (9–40 mm. Hg). The blood of 6 acutely adrenalectomized dogs consistently showed pressor activity after hemorrhage. Many of the responses were renin-like, persisting 10 to 30 minutes; others of equivalent magnitude approached the duration of reactions to angiotonin. With one exception, renal venous blood gave greater responses than corresponding arterial blood. Renal origin was demonstrated in 3 adrenalectomized, nephrectomized dogs by the absence of pressor reactions from arterial blood even with lethal hemorrhage.

That nephrectomy impairs the ability to maintain arterial blood pressure was demonstrated in 24 experiments. In one series the average blood loss required to lower the blood pressures to 30 mm. Hg in nephrectomized and sham-nephrectomized animals was 35.2 and 47.7 cc. per kgm. respectively. [*Aided by a grant from the American Philosophical Society.*]

Respiratory reflexes from the limbs. J. H. COMROE, JR. (by invitation) and CARL F. SCHMIDT. *Laby. of Pharmacology, Univ. of Pennsylvania.* In anesthetized dogs active movements produced by electrical stimulation of lumbar anterior spinal roots, as well as passive movements of vascularly isolated limbs, caused hyperpnea manifested chiefly by increase in rate and abolished by denervation (confirming Harrison, Arch. Int. Med. **50**: 690, 1932). Similar acceleration followed simple flexion of one knee. Since hyperpnea produced by any of these means disappeared after procainization of periarticular structures (but not after removal of the leg muscles or procainization of the synovial membrane) end organs involved appear to be propioceptors in ligaments and tendons about the joint. A similar reflex exists in normal men but both rate and depth of breathing are increased; of 32 subjects, 22 showed increases of more than 30 per cent in minute volume (maximum increase 95 per cent) during passive movement of one leg. Spinal anesthesia in 5 subjects markedly decreased or abolished this response.

In addition to this proprioceptor reflex system chemically sensitive receptors also exist in the limbs of dogs and cats. These respond to acids, KCl, phosphate buffers (pH 6.4 or less) and certain products of muscular contraction, but are not stimulated by alterations in blood pO_2, pCO_2, or pH compatible with life, by complete anemia, or (in normal men as well as anesthetized animals) by muscular contraction during total ischemia. Proprioceptors definitely contribute to hyperpnea of muscular exercise but chemosensitive system appears to be activated only by changes outside the physiological range.

The rôle of pressoreceptors in the regulation of blood pressure in the rabbit. R. E. CONKLIN and T. H. SIMISTER (by invitation). *Vassar College, Poughkeepsie, N. Y.* In the rabbit, afferent impulses causing reflex adjustments of the circulation to postural changes, are known to originate in vagus and aortic nerve endings, the carotid sinus and the Pacinian corpuscles of the mesentery. The effects of eliminating these afferent impulses have been studied. There was confirmation of the earlier results of Conklin and Dewey (Am. J. Physiol. **133**: 244, 1941), who found that elimination of vagus, aortic and carotid sinus nerve impulses had little effect upon the normal compensatory response of the rabbit to tipping to the foot-down position.

In addition, the elimination of splanchnic-borne afferent impulses by cutting the splanchnic nerves, has been tried. Although the blood pressure falls markedly when this is done, there is still at least a partial compensatory response during the tipping, and after the return to the horizontal position the blood pressure is often higher than before. In three rabbits whose splanchnic nerves alone were cut, the mean blood pressure at 60° tipping was 83 per cent of the horizontal value, whereas before cutting these

nerves the 60° tipping caused a drop to 74 per cent of the horizontal value. It seems probable that these are other diffuse sources of afferent impulses enabling the rabbit to compensate for the adverse effect of gravity on the circulation.

Calcium and potassium reversal effects of the perfused turtle heart. HELEN C. COOMBS. *Division of Industrial Hygiene, Delamar Inst. of Public Health, Columbia Univ., New York.* (Read by title.) In continuation of an earlier study (Coombs and Pike, Am. J. Physiol. **133:** 245, 1941) perfusion of the hearts of thirty-six turtles with Ringer's solution, lacking respectively calcium and potassium, was done in the late winter and early spring (February, March and April).

It was found that the turtle heart at this period of the year is relatively insensitive to lack of potassium in the perfusion fluid. In Ringer's solution lacking potassium it took from 8 to 25 minutes to reduce the magnitude of the contractions of the heart to half the control size, the rate remaining the same. Lack of calcium in the perfusion fluid reduced the contractions to half their original height in from 5 to 15 minutes. In both cases, when the hearts had been deprived of the control amounts of calcium or potassium in the perfusion fluid for several minutes, it took smaller amounts of the antagonistic ion to arrest the heart than it would in normally perfused controls. The addition of 5 cc. of M/100 KCl under conditions of calcium deprivation stopped the heart in systole, where a heart perfused with control Ringer's would stop in systole only after the addition of 2 to 5 cc. of M/10 KCl. Moreover, in the heart perfused with potassium-less Ringer, the addition of 3 to 10 cc. of M/100 $CaCl_2$ stopped it in diastole. This did not occur on addition of calcium to hearts perfused with control Ringer solution. It would appear, as in other physiological relationships, that the imbalance of the ions rather than their actual concentrations, produces this reversal.

Some effects of salt and water ingestion. OTIS M. COPE and FREDERICK M. ALLEN. *Dept. of Physiology and Biochemistry, New York Medical College, New York City.* In a paper before the previous meeting of this Society (April 1940), Allen and Cope presented observations showing that in dogs the ingestion of sodium chloride or water, or especially the two together, produced *a*, elevation of the femoral and brachial blood pressure as determined by auscultation; *b*, measurable transitory increase of the size of explanted kidneys. This latter effect was attributed to hyperemia, and indicates that the kidneys are subject to gross alterations of volume in the course of their diurnal functional changes. Controls showed that these results are not due to bulk of ingested material, or to digestion; also corresponding effects from protein were slight or doubtful. The present paper deals with continuation of observations along these lines.

The determinants of renal blood flow in hypertension. A. C. CORCORAN and IRVINE H. PAGE. *Lilly Laby. for Clinical Research, Indianapolis City Hospital, Indianapolis.* Measurements of effective renal blood flow per unit of functioning tubular mass (Tm_D) in 42 patients suffering from essential and malignant hypertension yield values which range from normal to grossly subnormal levels. Calculations of renal vascular resistances show that effective renal blood flow in hypertension is inversely proportional to increased efferent arteriolar resistance. Increased afferent resistance also participates, though in smaller measure, in determining the level of renal blood flow. Since blood flow through organs is proportional to arterial pressure and inversely proportional to resistance, the ratio $\frac{\text{mean arterial pressure}}{\text{renal vascular resistance}}$ was calculated. Correlation of this value with effective renal blood flow yielded a correlation coefficient (r) of 0.8. The relative level of effective renal blood flow in hypertension is therefore directly proportional to increased arterial pressure and inversely proportional to increased vascular resistance.

Hypoproteinemia, commonly present in these patients, increases filtration fraction with reference to intraglomerular pressure and efferent arteriolar resistance, while increased arterial pressure and anemia raise the level of renal plasma flow in relation to renal vascular resistance. Calculation of intrarenal hemodynamics in terms of relative blood flow, renal resistances and arterial pressure are therefore more informing in hypertension than the determinations of relative effective plasma flow and filtration fraction.

Our observations indicate that the level of renal blood flow in clinical, as in experimental hypertension, is not in itself an index of the origin or course of the disease.

The renal pressor system as an index of species relationship. A. C. CORCORAN, O. M. HELMER and IRVINE H. PAGE. *Lilly Laby. for Clinical Research, Indianapolis City Hospital, Indianapolis.* The pressor action of human renin in various mammalian species and the pressor inactivity of hog and beef renin in human beings have been considered evidences of renin-specificity, although the nature of the renal pressor system (renin, renin-activator, angiotonin) leads to the conclusion that the specificity is probably not that of renin, but of renin-activator. Testing this view, human renin was found to yield equal pressor activity (angiotonin) on incubation with the plasma (renin-activator) of various mammals, including man, baboon and monkey. Plasmas of

man, baboon and monkey do not yield angiotonin on incubation with the renin of hogs, sheep, rabbits, dogs or cats. Mammalian renin is therefore not specific as between species, but mammalian renin activator has broad specificity, in that in man, baboons and monkeys it differs from that of other mammals. Incubation together of hog renin and human renin-activator did not inhibit formation of angiotonin from dog plasma, subsequently added to the mixture. Failure of interaction in such cases is, therefore, complete and does not depend upon partial interaction or enzyme absorption without angiotonin formation.

Hen renin does not interact with mammalian renin-activator, nor does hen renin-activator react with mammalian renin. Both renin and renin-activator therefore differ from their mammalian counterparts in this species. Studies on reptiles, amphibians and fishes will be reported.

The evidence at hand indicates that the renal pressor system is widely distributed and that it provides a new broad index of species relationship.

Hepatic blood flow and glucose output in unanesthetized dogs. LATHAN A. CRANDALL, JR. and ALYS LIPSCOMB (by invitation). *Univ. of Tennessee, Memphis.* A new principle for the determination of hepatic blood flow has been applied to angiostomized (London cannula) dogs. The urea output in the urine is determined at hourly intervals for four hours; hourly determinations of the rate of liberation of urea into the blood stream by the liver (in mgm. per 100 cc. of blood) are made simultaneously. A simple calculation gives the value for total hepatic blood flow, after urine urea output has been corrected for any change in arterial blood urea level during the period. The figure so obtained is an average for the four hour period, and does not take into account fluctuations that may have taken place, but the usual constancy of hepatic urea output in terms of milligrams per 100 cc. of blood and of the rate of urea output in the urine indicate that fluctuations which might seriously affect the calculations are rare. The values for blood flow are in accord with those obtained by others using entirely different methods.

The determinations of hepatic glucose output in milligrams per 100 cc. of blood (glucose and urea both being determined on samples of portal venous, hepatic venous, and arterial blood) may be used to calculate total hepatic glucose output in grams per kilo per hour. The glucose output has never exceeded 0.16 gram per kilo per hour, which is less than the amount (0.25 gram per kilo per hr.) needed to maintain the blood sugar of the hepatectomized dog but agrees well with other reports of the hepatic glucose output of the anesthetized dog.

A revised frequency-map of the guinea-pig cochlea. ELMER A. CULLER. *Laby. of Psychology, Univ. of Rochester, Rochester, N. Y.* When the tympanic cavity of the guinea pig is exposed on the ventral side, the cochlea becomes accessible for study without injury to any part of the acoustic mechanism. By moving a sharp wire-electrode over the outer surface of the cochlea, one can measure and correlate the magnitude of potentials generated at various points inside it. In 1935, I published such an electrophonic map, of which the present study is a revision; based indeed upon the same technical and statistical procedures and postulates, but with greatly improved equipment. The distribution varies somewhat from ear to ear, but the mean locations agree satisfactorily with those reported in the earlier chart. The first apical turn, ventral aspect, provides loci for about 100 cycles (medial area), 200 cycles (central area) and 300 cycles (lateral area); the second turn likewise extends from about 500 to 1000 cycles; the third turn from about 2000 to 3000 cycles. Whatever may be the exact mechanism by which cochlear microphonics are generated, it is clear that they do not arise uniformly nor haphazardly along the cochlear tube, but follow instead a linear sequence in step with the impressed frequency. It is also noteworthy that the lower frequencies (e.g., 75, 100, 150 cycles) are localized as consistently as are the higher frequencies around 2000 cycles.

Dr. J. D. Coakley, Dr. Karl Lowy and N. Gross have collaborated in this study.

Thyrotropic hormone assay in the tadpole. SAVINO A. D'ANGELO (by invitation), ALBERT S. GORDON (by invitation) and HARRY A. CHARIPPER. *Dept. of Biology, Washington Square College of Arts and Science, New York Univ., New York City.* It has already been shown (same authors, 1941) that total starvation of tadpoles prior to a critical developmental stage suppresses pituitary function and results in thyroid atrophy with consequent metamorphic stasis. In the present experiments a quantitative study of pituitary replacement was made by administering various amounts of a thyrotropic extract (Antuitrin T), previously standardized against the immature guinea pig, to totally starved Rana pipiens tadpoles at the 4–6 mm. hind limb stage. It was found that 4 pleuro-peritoneal injections (daily or alternate days) of 0.05 cc. of a solution containing full strength extract or dilutions of 1–5, 1–50, 1–200, 1–400, and 1–800 of the extract were capable of causing greater weight loss (in respective order) in tadpoles than in water-injected controls. The weight loss was accompanied by unmistakable metamorphic changes in the head and body as well as increase in hind limb length. Emergence of forelimbs occurred with the lower dilutions after the 7th injection. The control animals, on

the other hand, made little or no progress in development. Thyroids of the thyrotropic-injected animals were activated as shown by increased vascularity, heightening of the epithelium, and colloid release. Thyroids of control tadpoles receiving injections of either water or solutions of glucose, glucosamine, cysteine, glutathione or thyroxine remained atrophic.

These results suggest that the "stasis" (starved, non-metamorphosing) tadpole is an exceedingly sensitive test object for the detection of thyrotropic hormone and that this sensitivity is of a greater order than that previously reported for either the immature guinea pig or the chick.

Excitatory and homeostatic effects on the electroencephalogram. CHESTER W. DARROW. *Inst. for Juvenile Research and Dept. of Physiology, Univ. of Illinois College of Medicine, Chicago.* By means of filters, integrated records of beta and alpha frequency potentials, respectively, are polygraphically recorded in parallel with concomitant palmar galvanic (sweating), systolic blood pressure and respiratory changes of human subjects. It is thus instrumentally confirmed that after stimulation decrease of alpha and increase of beta tend to be associated with autonomic effects indicative of excitation. It is further shown that the presence of a rise in blood pressure may exert an opposite or homeostatic influence which tends to keep the electroencephalogram constant even in the presence of excitation. In the absence of stimulation a rise in blood pressure tends to be associated with an increase of alpha and a decrease of beta activity—converse effects with a fall in blood pressure. The possible relation of these findings to the known effects of cerebral excitation and of homeostatic regulation on the cerebral blood vessels is considered.

Direct measurement of brain oxygen concentrations with a platinum electrode. P. W. DAVIES and FRANK BRINK, JR. (introduced by D. W. Bronk). *Johnson Foundation for Medical Physics, Univ. of Pennsylvania, Philadelphia.* A stationary platinum electrode has been used to measure local oxygen concentrations in the cat brain. Oxygen in the vicinity of the electrode tip is electrolyzed, and the current which flows at some given time after the circuit is closed is proportional, under the specificed conditions, to the oxygen concentration in the solution.

At first electrodes coated with glass down to the tip were tried, but these were unsatisfactory because they were too variable. Movements of the electrode and irregular convection currents caused short period variations in the current, while progressive contamination of the electrode surface caused variations from day to day. A more fundamental difficulty was that the electrode current is proportional to the diffusion coefficient of oxygen as well as to concentration, so that the absolute oxygen concentration cannot be determined unless the diffusion coefficient in the tissue is measured separately.

These difficulties have been avoided by using a recessed linear diffusion electrode. The platinum wire (176 micron dia.) is sealed inside a glass tube of the same inside diameter, with the end of the wire 1 mm. away from the orifice. The cylindrical cavity is filled with an agar salt solution. This electrode has proved to be very constant in its behavior, and since the electrolysis takes place in a medium of unvarying diffusion coefficient, the current is a direct measure of the oxygen concentration.

Measurements of tissue oxygen concentration made by this method will be presented in other communications at these meetings.

The production of experimental polycythemia, by the daily administration of epinephrine or posterior pituitary solution. JOHN EMERSON DAVIS. *Dept. of Physiology and Pharmacology, Univ. of Arkansas School of Medicine.* (Read by title.) We have reported previously (1941) that an experimental polycythemia may be produced in dogs and rabbits by the daily administration of certain doses of ephedrine. The results were attributed to increased erythropoiesis induced by hypoxia of bone marrow through diminution of its blood supply, as a result of the vasoconstrictor action of ephedrine. The present work was undertaken to see whether other vasoconstrictor drugs would also produce polycythemia.

The daily subcutaneous injection of 5 to 10 units of solution of posterior pituitary into one splenectomized and 2 normal dogs resulted in significant increases (15 to 20 per cent) in their basal erythrocyte numbers within 9 to 14 days. Hemoglobin percentages increased correspondingly, but the total leukocyte counts did not change significantly.

The red cell counts of 2 splenectomized and 4 normal rabbits were increased by 10 to 20 per cent by the daily subcutaneous injection of 0.5 to 2.0 units of posterior pituitary for about 15 days. The counts returned to normal within 10 to 15 days following cessation of drug injections.

The daily subcutaneous injection of 0.5 to 2 mgm. of epinephrine hydrochloride into one splenectomized and 2 normal dogs caused increases of 12 to 20 per cent in their basal erythrocyte numbers within 10 to 15 days. The hemoglobin percentages increased correspondingly while the total leukocyte counts remained fairly constant.

Potassium salt hemolysis. HUGH DAVSON. *Dalhousie Univ., Halifax, N. S.* Dog erythrocytes hemolyse in isotonic solutions of potassium salts, the rate and final degree of hemolysis varying

with the anion of the salt. When placed in an isotonic potassium solution the cells of the suspension separate themselves functionally into two groups: *a*, one which gains potassium rapidly and loses sodium slowly, and *b*, one which loses sodium rapidly and gains potassium slowly. Group *a*, consists of the cells which hemolyse whilst those in group *b* do not. This separation is determined by three main factors: 1. The relative values of P_K (permeability to potassium) and P_{Na}: vary among the cells, so that there is a group in which P_K is greater than P_{Na}: and one in which P_{Na}: is greater than P_K. 2. The swelling of the cells progressively reduces P_{Na}. 3. The shrinking of the cells progressively reduces P_K. The relative sizes of groups *a* and *b* under any conditions depend on the average initial values of P_K and P_{Na}; if P_K is initially very much greater than P_{Na} the proportion of *a* is large and the final degree of hemolysis is large. P_K and P_{Na} depend on the Na:K concentration ratio of the medium, being high when the ratio is small and vice versa. It is concluded that the hemolysis of the dog erythrocyte in isotonic solutions of potassium salts is an osmotic phenomenon due to the penetration of potassium.

Blood volume of the newborn in relation to early and late clamping of the umbilical cord. Q. B. DeMarsh (by invitation), W. F. Windle and H. L. Alt (by invitation). *Northwestern Univ. Medical School, Chicago.* One or two blood volume estimations were made on 35 infants by the Evan's blue method and the Evelyn photoelectric colorimeter. The cord was clamped immediately after birth in 17; clamping was delayed until the placenta had separated in 18. All deliveries were without narcosis. Ninety cubic centimeters or more of blood (averaging 107 cc.) was collected from the severed placental end of the cord in the former; none was obtainable from the latter because uterine contractions had transfused the placental blood into the newborn. Blood volume estimations were made 15 minutes to 3 hours after birth and/or on the third day.

There were no significant differences between day of birth and third day values. The average total volume in those retrieving placental blood at birth (24 experiments) was 361 cc. or 11.8 per cent body weight. The hematocrit was 60 per cent, and the plasma volume was 138 cc., or 4.5 per cent of body weight. After depriving the newborn of 107 cc. placental blood, the average total volume was 301 cc., or 9.6 per cent body weight. The hematocrit was 51 per cent and was equal to the hematocrit of the umbilical cord blood. The plasma volume, 140 cc. or 4.5 per cent of body weight, was not altered. Differences were statistically significant.

Rapid adjustment of plasma volume accompanied by hemal concentration occurs as the physiological placental transfusion takes place. This increases tissue fluid and raises the red corpuscle count and hemoglobin value of the newborn's blood.

The propagation of the pulse in the hand arteries. John B. Dillon (by invitation) and Alrick B. Hertzman. *Dept. of Physiology, St. Louis Univ. School of Medicine.* It was previously shown that in the propagation of the pulse wave distal to the radial artery, certain changes in form occurred by the time the pulse wave reached the minute finger pad vessels. It was inferred, at that time, that these changes in the contour of the pulse wave took place in the minute pad vessels. This point has been restudied by recording the pulse waves in the digital artery, at the proximal interphalangeal joint, and finger pad, with the photoelectric plethysmograph.

In normal resting subjects, the crest time of the digital artery volume pulse is less than that of the finger pad volume pulse, except in occasional cases where the crest times are equal. In the group studied, the normal digital artery crest time, varied between 0.09 and 0.12 second. These values are comparable to those previously reported for the radial artery (0.08–0.13 sec.). Also, the digital artery pulse very closely resembles the radial pulse. The form of the digital artery volume pulse is unaffected by arteriolar constriction in the finger although the pad pulse may be altered. These data indicate that in the normal subject the pulse wave is propagated unchanged in the hand arteries at least as far as the digital artery.

The effects of hypertension, arteriosclerosis and other peripheral vascular diseases, on the propagation of the pulse in the small arteries, are being studied. [*Aided by the Burgess Battery Company.*]

The effects of anoxemia, hypercapnia, acidosis and alkalosis upon the threshold of reactive hyperemia in human skin resulting from local ischemia. Joseph R. DiPalma (introduced by George B. Ray). *Dept. of Physiology and Pharmacology, Long Island College of Medicine, Brooklyn, N. Y.* By applying weights to the ventral surface of the forearm two end-points may be obtained. One, the stimulation or *threshold time* equals the time in seconds of application of a weight required to produce a reactive hyperemia of even intensity and localized to the stimulated area; the other known as the *clearing time* equals the time in seconds required for the reactive hyperemia to fade to the color of the surrounding skin. The apparatus, technique, individual and seasonal variations are adequately described in another communication (Am. Heart J., in press, 1942).

In anoxemia (rebreathing into a tank with removal of CO_2) the threshold and clearing times

rise progressively as the volume percentage of O_2 in inspired air falls. In hypercapnia (rebreathing into a tank) there is no change in the threshold time while the clearing first rises then falls as the CO_2 tension of inspired air rises. In acidosis (ingestion of NH_4CL) and in alkalosis (ingestion of Na_2CO_3) there is a direct relationship between the sum of urinary total acidity and urinary NH_3 and the threshold and clearing times. Obviously there is a decreased sensitivity of the small skin vessels to local ischemia in the former and an increased sensitivity in the latter.

Such a quantitative approach to the responses of the smallest blood vessels in relation to alterations of the milieu intérieur provides a new means by which this part of circulation may be studied in systemic disorders among which are peripheral vascular disease and diabetes mellitus. [*Supported by grants from the Josiah Macy, Jr. Foundation and the Committee on Endocrinology, National Research Council.*]

The physiological significance of compensatory adrenal atrophy. CHRISTIANE DOSNE (by invitation) and HANS SELYE. *Dept. of Anatomy, McGill Univ., Montreal, Canada.* Experiments on albino rats indicate that continued treatment with high doses of desoxycorticosterone acetate (D.C.A.) markedly inhibits, but does not completely prevent the adrenal cortical response during the alarm reaction. As a result of this inhibition the resistance of D.C.A. pretreated animals to nonspecific noxious agents is actually sub-normal since D.C.A. itself does not raise resistance but apparently interferes with the production of those corticoid hormones which increase general resistance. This view receives further support by the observation that D.C.A. pretreatment interferes with the normal glycemic response to nonspecific noxious agents, probably because it disturbs the production of carbohydrate active corticoids.

We conclude that overdosage with one of the compounds produced by an endocrine cell (in this case D.C.A.) can interfere with the production by the same cell of other hormonal compounds (in this case carbohydrate active corticoids such as corticosterone, etc.). As a result of this interference symptoms of overdosage with hormones produced by a certain endocrine cell type may coexist with signs of deficiency in the hormone production of that same cell.

Is there rennin in human gastric juice? LOUIS B. DOTTI and ISRAEL S. KLEINER. *Dept. of Physiology and Biochemistry, New York Medical College, Flower and Fifth Ave. Hospitals, New York.* It is well known that pepsin, as well as other proteolytic enzymes, has the power of clotting milk. Using milk buffered to pH 5.0 as a substrate, the clotting power of a fluid may be used as a measure of the pepsin + rennin content. (Barowsky, Tauber and Kleiner, Am. J. Digest. Dis. and Nutrition **4**: 229, 1937.) It has been shown (Tauber and Kleiner, J. Biol. Chem. **104**: 259, 1934) that if pepsin and rennin are mixed and incubated the rennin will be rather rapidly digested, as evidenced by diminution in the milk-clotting power. We have used this phenomenon to ascertain whether rennin exists in human gastric secretion.

Gastric fluid, obtained from adult humans, was incubated at 38°C. for varying periods up to 48 hours. The clotting power was determined before and at intervals during incubation. In all cases it remained constant. Addition of "scale" pepsin to some of these gastric samples resulted in mixtures which also had constant clotting power on incubation. On the other hand, when commercial rennin was added to similar gastric samples the total clotting power diminished to levels equivalent to the original strengths.

The conclusion is obvious that the clotting power of adult human gastric juice is due to pepsin and HCl; and the assumption is warranted that human gastric juice contains no rennin. This harmonizes with results obtained by other investigators using other methods.

Further ballistic studies on cardio-aortic hydraulic models. PHILIP DOW and W. F. HAMILTON. *Univ. of Georgia School of Medicine, Augusta.* The ballistocardiographic method, while by far the simplest for cardiac output determination, is still not without uncertainties. Experiments are being continued to determine to what extent the record is a reliable index of the stroke volume, and just what hydraulic factors are directly responsible.

For want of more precise information on such matters as curves of cardiac ejection and aortic velocity in man, and the normal range of their variation, certain fundamental assumptions were necessary in the development of the formulas currently employed. Evidence in the literature has cast some doubt on their validity.

Experiments on models, reported last year, have been extended and supplemented. A nearly simultaneous filling of all segments of the artificial aorta has made possible a great reduction in the aortic standing waves and their contribution to the ballistic record, with a concomitant clarification of the effect of the ejection alone. Methods have been devised to obtain independent control of the time relations between initiation, maintenance, and waning of the cardiac ejection as well as the gross stroke volume.

Results so far obtained suggest that, even within physiological ranges, other factors than the stroke volume may produce marked quantitative changes in the ballistocardiogram without

significantly altering its characteristic form. A somewhat modified theoretical approach indicates a possible reconciliation between the parallelism of ballistocardiogram and stroke volume on the one hand and, on the other, the previously reported deviations therefrom.

Cerebellar action potentials following proprioceptive and exteroceptive afferent stimulation in the rat. ROBERT S. DOW and ROBERT ANDERSON (by invitation). *Dept. of Anatomy, Univ. of Oregon Medical School, Portland.* (Read by title.) Previous attempts to obtain action potentials from the cerebellar cortex following physiological stimulation of afferent endings (R. S. Dow, J. Physiol. **94**: 67, 1938) had been unsuccessful in the cat. In rats under light nembutal anesthesia cerebellar action potentials were obtained when both proprioceptive and exteroceptive endings were stimulated. For the former tendons of the quadriceps femoris and triceps muscles were tapped after the remainder of the limb had been denervated. Cerebellar action potentials were also produced by lightly flicking the animal's hair on the back or extremities. The potentials were eliminated by decerebellation and were still present in decerebrate animals.

The responses from moving the hair (exteroceptive stimulation) were most marked in the culmen. Action potentials of lower amplitude were obtained from the pyramis, lobulus simplex and occasionally from the lobulus ansiformis and lobulus paramedianus. The vermian responses occurred following a latency of 31 to 34 mil. sec.

The responses from the tendon taps (proprioceptive stimulation) were restricted to the pyramis and uvula in most experiments. Occasionally slight responses on the culmen were obtained from stimulation of the triceps muscle. The latency for response from stimulation of the triceps and the quadriceps femoris was 16 to 18 mil. sec. No significant differences were detected in the responses from stimulation of topographically different parts.

Lipocaic and insulin sensitivity. LESTER R. DRAGSTEDT, J. GARROTT ALLEN (by invitation), FREDERICK M. OWENS, JR. (by invitation) and C. VERMEULEN (by invitation). *Dept. of Surgery, Univ. of Chicago, Chicago, Ill.* The development of lipocaic deficiency in depancreatized dogs is characterized among other things by a progressive decrease in insulin requirement and glucose excretion. When the liver becomes markedly infiltrated with fat, severe hypoglycemia with convulsions may be produced by a fraction of the amount of insulin originally well tolerated. This sensitivity to insulin is demonstrated by the profound fall in the blood sugar produced by as little as 2 units of insulin and may be employed as an additional criterion of lipocaic deficiency.

Emetic responses of glycosides as evidence of their cumulation and synergistic action in the central nervous system. MELVIN DRESBACH. *Harrison Dept. of Surgical Research and the Dept. of Physiology, School of Medicine, Univ. of Pennsylvania, Philadelphia.* In extending the work in which it was pretty definitely shown that individual glycosides can induce vomiting (in surviving animals) after all known afferent nerve paths connecting the trunk and brain had been severed (Am. J. Physiol. **133**: P. 264, 1941), not only have the abortive or incomplete, but none the less certain, emetic responses been elicited in further experiments under those conditions but other results of interest have been obtained. It has been observed that the persistence of the emetic action of the previously used glycosides, given in subliminal doses, and the consequent cumulative effects can be brought out just as certainly in these operated animals as in the intact ones; and emesis can be easily produced by synergistic action of these bodies under the same circumstances. An aglycone (strophanthidin) played an important part in the experiments (presently to be published in full), which strongly suggest that the processes here mentioned have their location, at least in part, in the central nervous system.

Permeability as a factor in the etiology of ulcers. ROBERT L. DRIVER (introduced by J. R. Murlin). *Dept. of Anatomy and Physiology, Univ. of Kentucky, Lexington.* The formation of ulcers by perfusing intestinal loops with a 2 per cent solution of pepsin in 0.1 N hydrochloric acid can be greatly facilitated by increasing the permeability of the intestinal mucosa.

Compounds which promote the absorption of insulin from the intestine cause an increase over controls of several hundred per cent in the number and severity of ulcers obtained by the perfusion method.

Suggested modes of action of some of these agents are given below. Hexylresorcinol is a powerful surface tension lowering agent and produces structural changes in the mucous membranes which modify permeability. Calgon (sodium hexametaphosphate) is a very effective agent in removing calcium from the mucosa. Calcium has a hardening effect on the mucosa and decreases the permeability of the membrane. The effect of methyl salicylate is attributed to its special irritating influence on the mucosa. Pinacol (tetramethyl glycol) probably changes the penetrability of pepsin by its particular molecular configuration.

Experiments are under way to determine whether a decrease in permeability of the mucosa will result in a decreased incidence of ulcers.

The amount of glucose formed from protein in

diabetics. D. R. DRURY. *Univ. of Southern California.* Experiments were carried out on phlorhizinized dogs and on depancreatized dogs to determine the amount of glucose that is produced per gram of protein utilized by the body. The classical D:N ratio of 3.65 found in phlorhizinized fasting dogs is concerned only with that fraction of the sugar formed from protein, which is excreted in the urine. It does not take into account the sugar oxidized by the tissues of the animal. When such animals are fed protein the D:N ratio drops, indicating an increase in utilization of glucose by the tissues. This fact makes these animals unsuitable for determining the true protein: glucose ratio.

The depancreatized dog does not show such a marked increase in glucose tissue utilization on feeding. In this preparation one can estimate the extra sugar resulting from fed protein since the amount used by the tissues is small and is easily corrected for. The glucose-nitrogen equivalence so determined is between 5 and 6.

Applying this higher D:N ratio to fasting diabetics one can account for both the glucose excreted in urine and that used by the tissues, from the body protein catabolized.

Deafness "neurosis" in the cat as a special form of disinhibition. SIMON DWORKIN, JUDITH O. BAXT (by invitation) and J. GROSS (by invitation). *Dept. of Physiology, McGill Univ., Montreal, Canada.* A requisite for the establishment of the alimentary-motor response in the cat is the suppression of excessive responses that characterize the early training. The eventual disappearance of the activity is due to a form of spontaneous extinction which we call 'interval' inhibition. Once established, this form of inhibition is strongly maintained, but nevertheless subject to many agencies that may affect the fundamental excitatory and inhibitory functions. The present report describes a characteristic change of behaviour that supervened in some animals which were previously conditioned to pure-tones and then made totally deaf by damage to the cochlea. There followed a striking state of hyper-activity manifested by much general body-movement, sudden orientations toward the food-carrier and irrepressible interval responses. This mass 'release' phenomenon could be perfectly controlled by the simple procedure of retraining the animals to visual or tactile stimuli; under these new conditions interval inhibition could be re-established in several hours, when the behaviour reverted to the normal. Just as easily the hyper-activity could be brought back by discontinuing the new, perceptible, stimuli.

The production of permanent hyperglycemia and glycosuria by the prolonged administration of insulin. SAMUEL ELGART (by invitation), NORTON NELSON (by invitation) and I. ARTHUR MIRSKY. *May Inst. for Medical Research, The Jewish Hospital, Cincinnati, O.* Persistent glycosuria and hyperglycemia have been produced in partially depancreatized dogs (33 to 66 per cent of pancreas removed), treated with protamine zinc insulin. Each animal, following an observational period to rule out operative diabetes, was placed on protamine zinc insulin in sufficient quantities to maintain the blood sugar level just above the hypoglycemic level. Food was given freely. In each instance, the insulin necessary to maintain the blood sugar level increased until from 30 to 45 units daily were required. In from 6 to 8 months of such treatment a persistent glycosuria and hyperglycemia became apparent. On omission of food and insulin, ketonuria and ketonemia ensued and death occurred within 10 days. Post-mortem examination revealed a small amount of pancreatic tissue in which the islet tissue was present as fibrosed areas or entirely absent.

The relation of these findings to the dangers of the prophylactic use of insulin will be discussed.

Effect of exercise on the coronary blood flow, pulse rate and blood pressure of trained dogs with denervated and partially denervated hearts. H. E. ESSEX, J. F. HERRICK, E. J. BALDES and F. C. MANN. *Division of Experimental Medicine, Mayo Foundation, Rochester, Minn.* Observations were made at varying intervals after the following procedures: 1, bilateral sympathetic ganglionectomy, from about the eighth costal interspace anteriorly including the stellate ganglion; 2, double cervival vagotomy; 3, right vagotomy followed by left vagotomy; 4, cardiac sympathectomy and right cervical vagotomy followed by left cervical vagotomy. Blood flow in the circumflex branch of the left coronary artery was observed by use of the thermostromuhr. Blood pressure was recorded, optically from cannulated femoral or carotid artery. Heart rate was observed electrocardiographically. The effects of exercise in animals with sympathectomized hearts were not essentially different from results obtained in animals with innervated hearts. In both series exercise produced increased coronary blood flow, pulse rate and blood pressure. The observations were made 34 to 124 days after sympathetic ganglionectomy. The effects of exercise were very similar in animals with complete cardiac denervation and those lacking only the vagi. Loss of the vagi affected cardiac acceleration profoundly. Vagotomized hearts increased only about 10 to 20 beats each minute with increments in the rate of work. This was true whether or not the sympathetics were present. In the absence of marked acceleration and elevation in blood pressure the coronary blood flow was little affected by exercise. In animals with vagotomized or totally dener-

vated hearts the coronary blood flow appeared to be influenced chiefly by the blood pressure.

The effect of thyroid parathyroidectomy on the cold blocking temperature of rat nerves. HAROLD N. ETS and R. D. TEMPLETON. *Loyola Univ. School of Medicine, Chicago.* Albino rats, used in other experiments, were killed with ether and a muscle nerve preparation made from the posterior muscles and sciatic nerve of each hind leg.

One of the preparations was placed in a modified balanced salt solution for thirty minutes and then blocked. The other preparation was placed directly in a moist chamber and blocked. The nerve was laid across a silver tube which was cooled by passing a cold solution through it. Temperatures were made by means of a thermocouple, one junction on the silver tube and the other in a water-ice mixture; readings were made by means of a galvanometer.

The end of the nerve distal to the muscle and silver tube was placed on an electrode and subjected to a stimulus from a five microfarad condenser charged to a voltage twice that of the threshold.

The results of blocking the nerves of twenty-two normal rats and sixty-four thyroidparathyroidectomized rats were analized statistically and indicate that the normal rat nerve blocks several degrees below the nerve of operated animals.

The relation of fractionate contraction in regions to the surface potential distribution. J. A. E. EYSTER, WALTER J. MEEK, RUTH E. CONKLIN (by invitation) and WARREN E. GILSON (by invitation). *Dept. of Physiology, Univ. of Wisconsin, Madison.* Simultaneous recording of calibrated unipolar curves along with a reference curve and either a myogram or differential curve, are made from a number of surface regions of the auricle. From the data obtained from these curves, maps of the potential distribution at various time instants during the QRS period are made and related to the onset of fractionate contraction in these regions. It is found that regions entering negative potentialinto contraction are not at a maximum positive or negative potential but lie between regions the potentials of which are respectively above and below the potential of the region which is starting to contract.

Exchange of potassium for ammonium in frog muscle. WALLACE O. FENN and LORRAINE F. HAEGE (by invitation). *Physiology Dept., Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.* Frog muscles were analyzed for potassium and for ammonium after immersion for various periods in a modified Ringer phosphate solution in which 1 of the sodium was replaced by ammonium. The concentration of ammonium inside the muscle continues to increase for several days when it attains a value 2 to 2½ times as great as that in the solution. About half of the ammonium is represented by a potassium loss. There were no significant or consistent changes in weight due to the ammonium. The accumulation of ammonium is increased by alkalinity, and decreased by the presence of potassium in the solution outside. Penetration of ammonium is only slightly diminished by killing of the muscle with chloroform or heat or by lack of oxygen. Ammonium accumulates, therefore, by exchange with potassium rather than by some expenditure of energy by the muscle.

An analysis of the oxygen consumption of yeast as related to cell division. KENNETH C. FISHER. *Dept of Zoology, Univ. of Toronto, Canada.* The quantitative examination of the effects of narcotics on the oxygen consumption of several types of cell leads to the conclusion that the respiration of these cells is actually composed of two distinct fractions, an "activity" and a "resting" metabolism. Inhibition of cell division in yeast by narcotics is closely correlated with inhibition of the activity metabolism. It is typical of such inhibitors that as their concentration is gradually raised, the activity metabolism is inhibited before an appreciable effect on the resting metabolism occurs.

If this interpretation of the quantitative effects of narcotics is substantially correct, then there is reason to believe that some substance must exist which will inhibit the resting metabolism first, as its concentration is raised. Furthermore, it is unlikely that such a compound would be found among the substances commonly recognized as narcotics. We have now to report that at least one inhibitor of this sort has been found. Sodium benzoate inhibits the oxygen consumption of yeast some 60–70 per cent before beginning to affect cell division.

Any serious doubt of the interpretation of the effects of narcotics seems, therefore, to be removed. Certainly in yeast the total oxygen consumption appears to be the sum of two or three fractions which can be inhibited more or less independently. Normal cell division requires the functional integrity of only one of these.

The absence of effect of thyroidectomy on the adult rhesus monkey. W. FLEISCHMANN, H. B. SHUMACKER, JR. (by invitation) and W. L. STRAUS, JR. (by invitation). *Dept. of Pediatrics, Surgical Hunterian Laby. and Dept. of Anatomy, Johns Hopkins Univ. School of Medicine.* In six adult rhesus monkeys total thyroidectomy had no effect on appearance, activity or the serum cholesterol level. In two of these animals B.M.R. was measured before and after operation and showed no change. These findings confirm older observations that thyroidectomy has no

effect on the appearance and activity of the rhesus monkey.

To determine whether all thyroid tissue had been removed, thyrotropic hormone of the pituitary was injected. By observing the effect on serum cholesterol and on creatine excretion in children we have been able to determine whether a patient has a thyroid capable of functioning effectively (Wilkins and Fleischmann). In these experiments creatine excretion was the most sensitive indication of thyroid effect. In the three thyroidectomized monkeys tested no effect on serum cholesterol and urinary creatine excretion was observed. There was, however, a marked response to thyroxin, both as regards creatine excretion and serum cholesterol. Unoperated animals showed a response both to thyrotropic pituitary hormone and to thyroxin. The lack of response to thyrotropic hormone in the thyroidectomized monkey can be interpreted as proof that no appreciable amount of thyroid tissue was left in the body at operation. This was furthermore proved by complete autopsy in five animals. In one of these complete serial sections were made of large amounts of tissue from mediastinum, neck and pharynx including the tongue. Thyroid tissue was entirely lacking in all of these.

The use of double work periods in the study of fatigue and the influence of caffeine on recovery. ELIOT FOLTZ (by invitation), A. C. IVY and C. J. BARBORKA (by invitation), *Depts. of Medicine and Physiology, Northwestern Univ. School of Medicine, Chicago.* Six subjects under controlled conditions of housing and diet were studied to ascertain whether a "double work period" might be more advantageous for the study of fatigue than the "single work period." A bicycle ergometer and a rather heavy load was used, 1235 KgM per minute with a pedalling rate of 54 RPM. Three times each week the subjects were worked to exhaustion, and after ten minutes of rest were worked to exhaustion again. *In general, as the length of the training period increases, the work output during the second period, the total work output for both periods, and the per cent recovery become less variable and approximately equal in variability; the work output during the first period remains the most variable of the four items of data.* The double work period in three of four of our subjects even after considerable training yielded three items of data less variable than the single or first work period.

Another advantage of the double work periods is that it permits a rapidly acting substance to be assayed during the training period, so that each subject may serve as his own control. Further, it permits a study of the effect of rapidly acting substances on recovery. It was found that the per cent recovery under our experimental conditions is inversely related to the work output of the first period; the correlation coefficient was 0.95.

Caffeine (0.25 gram) intravenously immediately after the first work period increased the per cent recovery in two out of four subjects. *Acknowledgment is made to the Abbott Foundation for rendering this investigation possible.*

Concerning the site of pentamenthylenetetrazol (metrazol) detoxification. GABRIEL FOURNIER (by invitation) and HANS SELYE. *Dept. of Anatomy, McGill Univ., Montreal, Canada.* The site at which metrazol is detoxified has hitherto been studied mainly with indirect methods using "liver poisons" or determining excretion in the urine (see review by Tatum and Kozelka, 1941) which led to rather conflicting results. That the drug is less toxic in the case of intra-portal administration than after injection into a peripheral vein may be due to loss of material during passage through liver capillaries without having any physiological significance.

In view of these uncertainties we decided to resume these studies using albino rats in which about 75 per cent of the liver tissue was surgically removed, (method of Waelsch and Selye, Arch. f. exper. Path. u. Pharmakol. **166:** 219, 1932). In addition to untreated controls we also had one group of bilaterally nephrectomized rats to reexamine the importance of renal excretion. It was found that the tolerance to subcutaneously injected metrazol was essentially the same in all three groups. Since similarly pheatectomized rats proved highly sensitive to drugs detoxified in the liver (Selye, H., J. Pharmacol. and exper. Therap. **71:** 236, 1941) it may be concluded that hepatic detoxification plays no important rôle in the case of metrazol. Furthermore, our findings confirm those of Dille and Seeberg (Proc. Soc. exper. Biol. and Med. **44:** 624, 1940) concerning the unimportance of renal elimination.

Increased total bile pigment output on a high fat diet. L. W. FREEMAN (by invitation), A. LOEWY (by invitation), A. MARCHELLO (by invitation), and VICTOR JOHNSON. *Dept. of Physiology, University of Chicago, Chicago, Ill.* The demonstration that, during absorption of a fat meal, thoracic duct lymph is hemolytic, raises the question whether red cells are actually destroyed in vitro in greater numbers on a high fat diet. Dogs with internal (and some with external) bile fistulae were alternately fed high fat diets for about two weeks, and then fed (calorically equal) low fat diets. The shift in diets was made several times on all dogs.

Daily total bile pigment excretion was measured, as an index of the rate of red blood cell destruction.

In five internal bile fistula dogs, the average daily total bile pigment output for all of the

periods of observation on the various diets showed that significantly higher values were obtained on the high fat diets than on low fat diets. Similar results were observed in two external fistula dogs. Two of the internal fistula dogs showed no significant differences.

It is concluded that red cell destruction probably proceeds at a faster rate on a high fat diet than on a low fat diet.

The effect of aluminum hydroxide ingestion upon iron retention by the anemic rat. SMITH FREEMAN. *Dept. of Physiology and Pharmacology, Northwestern Univ. Medical School, Chicago.* Earlier observations on Mann-Williamson dogs suggested the possibility that aluminum hydroxide might reduce iron absorption from the intestine. More specific information has been obtained on this question by determining the effect of aluminum hydroxide ingestion upon the retention of an iron supplement by anemic rats.

Litters of weanling rats were continued on a milk diet uncontaminated by iron until the hemoglobin content of the blood was approximately 2 grams per 100 cc. At this time some of the animals were killed and the total iron content of each carcass was determined. The remaining rats were divided into 2 groups of 10 rats each with an equal distribution, according to weight, sex and litter, in each group. For the remainder of the experiment each animal received a daily supplement consisting of 0.25 mgm. of iron as ferric chloride and 0.05 mgm. each of copper and manganese, fed as their sulphates. In addition, 5 cc. of 5 per cent aluminum hydroxide was mixed with the daily allowance of milk for each rat of one group. The rats were continued on this regimen until the hemoglobin content of one group had reached approximately 8 grams per 100 cc. of blood. At this time all of the animals were killed and the total iron content of each carcass was determined. Without exception, the animals receiving aluminum hydroxide contained less iron at the end of the experiment than did the control group. The average iron content per 100 gm. of rats was 2.25 mgm. for the aluminum hydroxide group and 3.42 mgm. for the control group.

The effect of other "antiacids" upon iron retention is being studied by the same technique.

Cardiac hypertrophy in experimental atheromatosis and its relation to the cholesterol content of the heart. L. FRIEDBERG and M. HURWITZ (introduced by L. N. Katz). *From the Cardiovascular Dept., Michael Reese Hospital, Chicago.* It has long been a controversial issue as to whether or not coronary artery disease per se could cause cardiac hypertrophy. Experimental production of coronary atherosclerosis in this laboratory by means of high cholesterol diets in rabbits has been shown to result in definite increased weight of the heart in the absence of associated valvular disease or hypertension. The possibility exists, however, that the increased weight might be accounted for entirely by the deposition of cholesterol in the heart muscle.

To determine whether or not this assumption is correct, a series of eight rabbits were subjected to high cholesterol diets and the hearts were quantitatively analyzed for cholesterol content by a standardized method. The results were compared with similar analyses on fourteen control rabbits. Although there was a definite increase in the amount of cholesterol, both in the free and esterized form, the increase was not sufficient to increase materially the weights of the hearts of the experimental rabbits. The conclusion, previously expressed that the increased heart weight is the result of hypertrophy of muscle fibers due to myocardial ischemia is thus confirmed. *Aided by the A. D. Nast Fund for Cardiovascular Research.*

On the physiological significance of the abnormally decreased or absent 17-ketosteroid excretion in Addison's disease, panhypopituitarism and myxedema. HARRY B. FRIEDGOOD. *Medical Clinic, Peter Bent Brigham Hospital, Dept. of Medicine, Harvard Medical School, Boston, Mass.* Determinations of the 24-hour 17-ketosteroid (17-KS) excretion were made on 10 patients with spontaneous or post-operative myxedema, in 2 cases of panhypopituitarism due to xanthomatosis and eosinophilic adenoma, respectively, and in 4 instances of Addison's disease. Simultaneous hydrolysis and extraction (CCl_4) were done in the Wolfe-Hershberg apparatus. Colorimetric determinations of the 17-KS excretion were carried out with the Zimmermann reaction (absolute alcohol technic) and the readings were made with the Evelyn photoelectric colorimeter. The 17-KS excretion was extraordinarily low in all of these cases; and, had the correction factor suggested by Fraser, Forbes, Albright et al. been applied in order to eliminate interfering color due to non-17-KS, many of these patients in all 3 groups would have shown no 17-KS excretion whatever. The current conception that the major portion of the 17-KS come from the adrenal cortex and only relatively small amounts are derived from the gonads leaves something to be desired in explanation of the low or zero values in myxedema. It is conceivable that a diminished or absent pituitary function could result in sufficient atrophy of the adrenal cortex to account for a markedly diminished 17-KS excretion; but that myxedema affects the adrenal cortex similarly is not clear. It cannot be argued that the lowered BMR in myxedema results in a general metabolic depression which affects the adrenal cortex adversely,

because adequate thyroid replacement therapy is without significant effect on the level of 17-KS excretion.

Influence of temperature on the stimulation of oxygen consumption of isolated rat brain cortex by 2-4 dinitrophenol. FREDERICK A. FUHRMAN (by invitation) and JOHN FIELD, 2ND. *Dept. of Physiology, Stanford Univ.* Administration of 2-4 dinitrophenol (DNP) to the anesthetized cat depresses the mechanism for chemical defense against cold, as indicated by decrease or abolition of shivering. Furthermore, in this animal, the calorigenic action of DNP becomes relatively greater as body temperature falls from 38° to about 33°C. (Hall, Crismon and Chamberlin).

To provide additional information on the influence of temperature on the action of DNP, we have investigated the percentage stimulation of oxygen consumption evoked by a given concentration of this drug in isolated rat cerebral cortex. Thermostat temperatures ranged from 10° to 42°C.

It was found that percentage stimulation of cerebral cortex respiration evoked by 0.75 mgm. per cent sodium 2-4 dinitrophenoxide increased as the temperature rose from 10° to 30°C., and diminished with further rise in temperature. The absolute value of the increase in the rate of oxygen consumption due to the presence of the drug increased from 10° to 35°C. and decreased with further rise.

On the assumption that the influence of temperature on DNP action is substantially the same for cerebral cortex and for the hypothalamic thermal centers, the results are in harmony with the hypothesis of Hall that abolition of the cold defense reactions by DNP may be attributable to the calorigenic action of the drug on the temperature centers. The fact that this action is relatively intensified with decrease in temperature within rather wide limits appears to be an additional argument in favor of this hypothesis. *Supported by grants from the Markle Foundation and from the Fluid Research Fund of the Stanford University School of Medicine.*

Phosphate exchange in resting cardiac muscle as indicated by radioactivity studies: II. ROBERT F. FURCHGOTT (by invitation) and EPHRAIM SHORR (with the technical assistance of GLADYS BREWER). *The New York Hospital, and the Dept. of Medicine, Cornell Univ. Medical College, New York City.* Previous radioactivity tracer studies on phosphate exchange in slices of dog cardiac muscle were extended to include a more detailed analysis of the state of inorganic phosphate (IP) in this tissue. This was accomplished by a preliminary incubation at 37.5°C. in oxygenated Ringer-m/75 phosphate, followed by prolonged washing in Ringer at 5°C., with chemical and activity analyses throughout the procedure.

The total IP was found to consist of a readily diffusible fraction IP_1 (30–40 per cent) and a difficultly diffusible fraction IP_2. Specific activity (radioactivity per gram phosphorus) of IP_1 after 30 minutes exposure to $P^{32}O_4$ equalled that of the medium IP; that of IP_2 was much lower and dependent on metabolism during exposure (20 per cent after incubation at 37.5°, 5 per cent after incubation at 5°C.).

The specific activity of phosphocreatin (CP) remained constant throughout washing, despite occasional decreases in total concentration, and was equal to that of IP_2. Both specific activity and concentration of adenylpyrophosphate (APP) remained constant during washing, but its specific activity was only about 65 per cent of that of CP.

The differences in specific activity and diffusibility of IP and IP_2 may indicate that the former is intercellular and the latter, intracellular. The greater specific activity of CP as compared with APP is not consistent with the prevailing view that APP is the donator of phosphate to CP, except on the basis that only one of the phosphates of APP is principally involved. The significance of the equality of specific activities of IP_2 and CP is being further investigated. *This study was aided by a grant from the Carnegie Corporation. The radioactive phosphorus was kindly supplied by Dr. J. H. Lawrence of the Radiation Laboratory, University of California, Berkeley.*

The response of single auditory nerve fibers to acoustic stimulation. ROBERT GALAMBOS (by invitation) and HALLOWELL DAVIS. *Dept. of Physiology, Harvard Medical School, Boston, Mass.* According to the place theory of action of the mammalian cochlea, each sound frequency at threshold excites a characteristic area of the basilar membrane, and that area is enlarged when the sound intensity is increased. This theory has been tested by isolating single auditory nerve fibers and determining their response to sounds of various frequencies and intensities.

By the use of microelectrodes the electrical response of single auditory nerve fibers in cats has been studied. Each fiber when isolated is excited by a narrow band of sound frequencies, and it discharges at threshold at a low frequency not obviously related to that of the sound. It follows increase in stimulus intensity by increasing its rate of discharge, and in this respect resembles other sensory nerve fibers. Frequencies adjacent to that to which the fiber responds at threshold elicit responses when the intensity level is raised, and very intense sounds an octave or more below have caused certain fibers to respond. These results are in accord with the place theory.

Additional material dealing with equilibration and adaptation will be discussed.

Cardiac conditioned reflexes to painful stimuli. W. HORSLEY GANTT. *Pavlovian Laby., Phipps Clinic, Johns Hopkins Univ.* Previously it has been shown in this laboratory that the conditioned reflex can be expressed by an exponential formula (Am. J. Physiol. **123**: 74, 1938) $CR = a + b\,(1 - e^{-cQ})$ in terms of secretion and quantity of food. The cardiac conditioned reflexes are also quantitatively related to the amount of food, so that a signal representing a large amount of food is accompanied by a greater acceleration than a signal for a small amount. Cardiac CRs to painful stimuli are also proportional to the intensity of stimulus ("motivation"). The latent period of the cardiac CR is short—within one or two heart cycles. The variations in cardiac CRs depend upon the individual, accounted for by *a*, *b*, *c*, of the above formula. Although the cardiac CRs have not yet been shown to bear an exponential relationship to quantity of unconditioned stimulus, they are like the secretory CRs quantitatively related to unconditioned stimulus, as regards both motor and secretory reflexes, involving defense and food.

Cardiac CRs can be used as quantitative measures of the normal CR as well as a gauge of emotional tension. When this becomes too great, the relations are chaotic, which is characteristic of psychopathology. The pattern of the change is constant for a given individual.

Maintenance of lactation in adrenalectomized rats. ROBERT GAUNT, E. C. KENDALL and W. J. EVERSOLE (by invitation). *Dept. of Biology, New York Univ., New York City, and Mayo Foundation, Rochester, Minn.* Previous work showed that desoxycorticosterone would not maintain lactation in adrenalectomized rats (Gaunt, 1941). Additional studies of a similar kind have been made with other steroids. The growth rate of suckling young served as a measure of lactation.

Compound A (dehydrocorticosterone), in doses of 0.5 mgm. per day, maintained a lactation that approached but did not reach normal.

Compound E (Kendall) in similar doses produced similar results when given either orally or parenterally.

Compound E, given in 1 mgm. daily doses, maintained a growth rate in suckling young that was normal for the first 17 days, but dropped slightly below normal around the twentieth day.

Whole adrenal cortex extract (Kendall) supported a near-normal lactation with 0.6 cc. daily. The amorphous fraction (Kendall), of a potency equal to that of the whole extract in maintaining adrenalectomized dogs, was of no benefit in doses of 0.6 cc. daily. A combination of 0.5 cc. of the amorphous fraction and of 0.5 mgm. of compound E daily gave effects no different than expected from the compound E alone.

We have studied further the possibility that desoxycorticosterone actually inhibits lactation in adrenalectomized rats. An apparent inhibitory effect can be seen in some but not all animals given up to 1 mgm. daily.

Progesterone in 2 mgm. daily doses probably had no beneficial and clearly no inhibitory effect on the milk secretion of adrenalectomized rats.

Adrenalectomized rats were found to be more sensitive than intact ones to the lactation-inhibiting effects of estrogen.

The transfer of water across the placenta of the guinea pig. ALFRED GELLHORN (by invitation) and LOUIS B. FLEXNER. *Dept. of Embryology, Carnegie Inst. of Washington, Baltimore, Md.* (Read by title.) Dilute heavy water (DHO) is physiologically indistinguishable from water (H_2O). The transfer of water across the placenta of the guinea pig has been studied at various periods of gestation using heavy water (DHO) as the tracer material. There is a ninefold increase in the rate of transfer of water across a unit weight of placenta from the 28th day of gestation to term. This is correlated with changes in the morphology of the placenta from hemochorial in early pregnancy to hemoendothelial in late gestation. The supply of water to a unit weight of fetus per hour can be correlated with the relative growth rate of the fetus. When the fetus is reproducing itself with great rapidity, the rate of supply of water to a unit weight of fetus is correspondingly great. As the fetus approaches term and the relative growth rate falls, the transfer of water per gram fetus per hour is commensurately low. Water moves across the placenta with astonishing speed. This is reflected in two observations. 1. A volume of water equal to about twice the weight of the fetus is transferred to the fetus across the placenta in one hour. This is true for fetuses weighing from one gram to one hundred grams. 2. The complete turnover of the water of the amniotic fluid occurs approximately once in every hour.

The influence of hypoglycemia and electroshock on the electroencephalogram. E. GELLHORN and M. KESSLER (by invitation). *Depts. of Physiology and Psychiatry, Univ. of Illinois College of Medicine, Chicago.* (Read by title.) Hypoglycemia induced by insulin (2 to 10 units/kgm. subcutaneously) in adrenalectomized rats causes a gradual diminution of alpha-waves and the appearance of large delta-waves. At later stages the alpha-waves disappear almost completely and the delta-waves are of lesser amplitude. If no glucose is injected the animals convulse and die. However, if during the preconvulsive state convulsions are produced by electroshock the E.E.G. returns, after a period a nearly complete silence and marked depression,

to an almost normal state characterized by good alpha potentials and spindles whose frequency is similar to that of the alpha potentials and whose amplitude is greater than that of the alpha-waves under control conditions. The rats appear normal although the blood sugar is very low. In the further course of the experiment, the E.E.G. may again show signs of hypoglycemia (delta-waves, no alpha potentials, no spindles) which can be offset by the injection of glucose.

The effects of lowered barometric pressure on man. CHALMERS L. GEMMILL. *School of Aviation Medicine, Naval Air Station, Pensacola, Fla.* In the routine training of aviation students, over three thousand individuals have been observed in a low pressure chamber at pressures corresponding to 18,000 feet without extra oxygen and at 28,000 feet with extra oxygen. The reactions of these men at the two altitudes will be discussed.

Excitation and membrane potentials of single muscle fibers. R. W. GERARD and JUDITH GRAHAM (by invitation). *Dept. of Physiology, Univ. of Chicago.* With one microelectrode inside a muscle fiber and the other outside the membrane (Graham, Carlson and Gerard abstract), electric stimuli (constant currents, condenser discharges, spike shocks) delivered in the direction from inside (anode) to outside (cathode) are effective, the reverse ones do not evoke a twitch. The time-intensity curve (inside anode) is the familiar one and quantitative data on transmembrane currents will be presented.

When a fiber is excited, through the nerve or at a distant region, the membrane potential falls. Conversely, the electric stimulus (applied across the membrane) which is required to elicit a response varies with the preexistent membrane potential.

These findings offer direct confirmation of the view that excitation results from a critical reduction of a preexistent membrane potential and that such depolarization is self-propagating.

Possible rôles of cH in neurophysiology. ROBERT GESELL, CHARLES R. BRASSFIELD and ELWOOD T. HANSEN (by invitation). *Dept. of Physiology, Univ. of Michigan.* Reflexogenic hyperpneas produced by stimulations of Hering's nerve were followed by after-hyperpneas (after-discharges) tending to vary inversely with the duration of stimulation (a significant diversion from spinal after-discharges [Sherrington] where superventilation is missing). Preventing overventilation (constant ventilation during pneumo-thorax) and administering CO_2 (chest intact) causes large increases of after-discharge which conforms with Sherrington.

This suggests the possibility that cH modifies nervous integration by gradation of longevity of acetylcholine wherever physiologically deposited. Miscellaneous observations and stimulations (sensory and motor nerves) support this theory. This intimate liaison reveals basic principles of nerve cell activation, for without altering temporal relations of impinging signals cH is capable of grading stimulations, theoretically through the sum-total of surviving acetylcholine miscellaneously deposited.

Acid-neurohumoral coordination disagrees with electro-temporal concepts of synaptic transmission. It conforms better with a more steady chemical or electro-chemical drive offered by the neurohumoral concept. CO_2 favors kindred phenomena, summation and after-discharge,—summation, by allowing accumulation, and after-discharge, by preventing destruction of acetylcholine. Waning after-drive continues as the most logical explanation of after-discharge (see Dale, Cannon, Gesell). Just as CO_2 favors summation, progressive alkalinization (continued Hering nerve stimulation) accomplishes "subtraction" i.e. a diminution of tidal-air. Temporary survival of acetylcholine thus becomes an asset rather than a liability. It permits elastic gradation of stimulating value of individual signals, an advantageous mechanism missing in electro-temporal concepts.

Acid-neurohumoral interplay may influence all physiological processes and also pathological manifestations where acetylcholine is involved e.g. mountain-sickness, tetany, coma, etc.

An acid-neurohumoral mechanism of gradation of stimulation. Demonstration of the principles on heart, striated muscle and intestine and a summary of experimental findings with original tracings. ROBERT GESELL, CHARLES R. BRASSFIELD, ELWOOD T. HANSEN (by invitation) and ARNOLD MASON (by invitation). (Demonstration.)

Differentiation of the effect of low O_2 and low CO_2 on the electrical activity of the cortex. F. A. GIBBS, W. G. LENNOX (by invitation) L. F. NIMS and E. L. GIBBS (by invitation). *Dept. of Neurology, Harvard Medical School.*) When normal subjects are rendered unconscious by breathing a mixture of two per cent O_2, five per cent CO_2, and 93 per cent N_2, no high-voltage slow waves appear in the electroencephalogram. The only change evident in the electrical record is a decrease in amplitude without gross evidence of slowing. This is in marked contrast to the change that occurs when consciousness is lost as a result of breathing low O_2 with no CO_2 added, for under these conditions high-voltage slow waves are a prominent feature of the record but studies on internal jugular and arterial blood indicate that such slow waves are the result of lowered CO_2 tension in the brain and not the result of cerebral anoxia.

The relation of fractionate contraction at various surface regions of the ventricle of the snapping

turtle to the surface potential distribution. WARREN E. GILSON (by invitation), RUTH E. CONKLIN (by invitation) and J. A. E. EYSTER. *Dept. of Physiology, Univ. of Wisconsin, Madison.* By the use of three direct coupled amplifiers and a three trace cathode ray tube, unipolar curves along with a reference curve and either a myogram or differential curve, are recorded from a number of surface regions of the ventricle. By dividing the calibrated unipolar curves intc equally spaced time instants and measuring the potentials at each time instant in all curves, a series of maps are obtained which show the potential distribution at the various time instants. The myograms or differential curves indicate the region or regions which are entering into contraction at each of these instants. It is found that a region entering into contraction is not at the time at a maximum positive or negative potential but in general lies between regions the potentials of which are respectively above and below the region which is starting to contract.

Striate and autonomic components of conditioned reflexes established under erythroidine and curare. EDWARD GIRDEN (Fellow, John Simon Guggenheim Memorial Foundation, at the University of Rochester, Rochester, N. Y.) (Introduced by E. A. Culler). *Brooklyn College, Brooklyn, N. Y.* Conditioned reflexes (CR) were studied in 25 dogs (2 to 6 mos. of age) subjected to varying dosages of 1 per cent water-solution of dihydro-*B*-erythroidine hydrobromide (supplied through the courtesy of Merck and Company, Rahway, N. J.) and curare injected intravenously. When required, artificial respiration was maintained. CR were established by paired combinations of light or bell with momentary shock applied to paw. Striated and pupillary muscle and blood pressure (BP) responses were recorded. The first two responses were taken by (16 mm.) motion picture technic and BP was measured from the common carotid artery by means of mercury manometer recordings on a continuous polygraph.

The form of the striated muscle-CR was a function of the dosage. The contractions became more attenuated as the myoneural block was increased. With mild doses, the reactions consisted of violent generalized activity akin to rage. Both striated and autonomic components of the drug-state CR have been retained as long as 22 days without intervening shock reënforcement. During this normal interval the CR was repressed and reappeared only when the drug-state was reinstated.

Tests with both curare and erythroidine showed that the action of the two drugs was identical in effecting this dissociation or cleavage of generalized emotional responses which had been previously reported for the isolated muscle under curare (J. Comp. Psychol. **23**: 261, 1937; Am. J. Physiol. **126**: P 472, 1939).

Blood changes following glucose, lactate and pyruvate injections in man. WALTER GOLDFARB, and ERNEST BEUDING (by invitation), *Bellevue Hospital and Dept. of Psychiatry, New York Univ., New York City.* We have attempted to estimate the interrelationship of intravenously injected glucose, lactate and pyruvate in humans. A large percentage of these products was retained in the body and presumably oxidized. The administration of glucose was associated with a rise of both lactate and pyruvate in the blood. The injection of d-lactate or r-lactate produced a rise of blood pyruvate. Pyruvate administration produced a rise of blood lactate and carbonyl compounds other than pyruvic acid. Neither lactate nor pyruvate injections caused any change of blood glucose. [*Through a grant by the John and Mary Markle Foundation and the William R. Warner Co. Research Fellowship Fund.*]

Effect of cobalt on anemia induced by x-rays in the guinea pig. ALBERT S. GORDON (by invitation), WILLIAM KLEINBERG (by invitation) and HARRY A. CHARIPPER. *Dept. of Biology, Washington Square College of Arts and Science, New York Univ., New York City.* (Read by title.) We have previously shown that cobalt exerts a beneficial influence on anemia produced by hemorrhage or benzol. In the present work, the effect of cobalt on the blood picture of guinea pigs subjected to x-rays was studied. Twenty-nine animals weighing 350 to 400 grams were given x-ray doses of 165.6 Roentgen units over a 4 minute period once weekly for 6 weeks. As a result of this treatment, the red cell count declined from 5.9±0.3 to 3.4±0.6 millions/cu.mm. and the white cell count dropped from 10.4±3.5 to 1.9±0.7 thousands/cu. mm. The reticulocytes decreased from 0.7+0.4 per cent to, in most cases, less than 0.1 per cent. Twenty of these animals were now given daily subcutaneous injections of 5–10 mgm. $Co(NO_3)_26H_2O$, the other 9 were left untreated. The treated animals revealed some signs of heightened erythropoiesis as evidenced by a rise in reticulocyte count from an average of 0.1 to 2.1 per cent and increased numbers of erythrogenic elements in the bone marrow sections and smears. The red and white cell counts of both the experimental and control animals, however, continued to fall, and the animals in both groups succumbed within 2–3 weeks. Death probably occurred as a result of the intense leucopenia present in all the animals. No x-ray exposure could be found which would produce an anemia and yet not affect the whites substantially. Injections of 0.25–1.0 mgm. nucleic acid made along with the cobalt in several of the animals did not affect the course of the anemia or leucopenia.

Extracellular fluid volume in man, and its relation to season. ARNOLDUS GOUDSMIT and LAWRENCE LOUIS (by invitation). *Dept. of Physiology, Univ. of Pennsylvania, Philadelphia.* Following the technic described earlier (Am. J. Physiol. **133:** 297, 1941) seventeen complete studies of total extracellular fluid volume were carried out on eight healthy male volunteers. Observations were made between October 1940 and July 1941. During this period the pertinent outside temperatures varied between 16 and 95°F. Both "bromide space" and "thiocyanate space" were determined.

These fluid spaces appeared rather constant for a given individual. A weighted average of all determinations for a given day was computed; it was found that this figure was reproducible for the same individual with an average standard deviation of ±1.2 per cent. After taking into consideration body weight, surface area, and serum protein concentration, a rather large inter-individual variability remained, which could not be correlated with age or habits of water and salt ingestion.

The average volume of extracellular fluih of seven individuals determined at the highest environmental temperature was 0.14 per cent (actually 24 cc.) higher than the one determined at the lowest temperature. This difference is entirely insignificant statistically. Thus increases in the volume of the circulating blood, itself a component part of the extracellular fluid volume, such as were observed to occur in the warm weather by Dr. Bazett and his co-workers might well be effected practically exclusively through transfer of water from other component parts of the extracellular fluid system. The support of the John and Mary R. Markle Foundation is gratefully acknowledged.

Membrane and injury potentials of single muscle fibers. JUDITH GRAHAM (by invitation), G. R. CARLSON (by invitation) and R. W. GERARD. *Dept. of Physiology, Univ. of Chicago.* Membrane potentials of single muscle fibers of the frog sartorius have been determined by inserting (micromanipulator) a capillary electrode (5–10μ outer diameter, containing isotonic KCl, resistance 10 megohms). The active and an indifferent electrode (micro or wick) connect through calomel cells with a modified Wynn-Williams balanced amplifier. Galvanometer sensitivity permits 1 mm. deflection for 0.3 mV. The electrodes are isopotential in Ringer and differ at most by 4 mV when moved about an uninjured muscle.

On insertion into a previously normal muscle fiber, the microelectrode registers 54 mV negative to an outside electrode. (Range 40 to 75, higher in winter; in one muscle, 20 per cent variation.) This membrane potential falls roughly exponentially, about 1 mV per minute for the first 15 minutes, per 10 min. for the third hour. The fall is not due to "healing" over the electrode, as shown by reinsertion, but to localized injury; and injury (by cutting, ether, or KCl which act differently) several millimeters distant does not hasten the fall.

The "injury" potential (measured within seconds) between a cut or chemically treated and an intact region is usually less than a third of the membrane potential. For example, an etherized muscle-end was 22 mV negative to the outside of a fiber at a distant point and 47 mV positive to the inside (sum = 69 mV membrane potential; directly measured, 67 mV). The curves of potential with distance, for both inner and outer sides of the fiber membrane, reach their asymptotes by 2 to 3 mm. from a cut. (Compare abstract, Gerard and Graham.)

Estrogens and phenolases. MARK GRAUBARD and GREGORY PINCUS. *Physiological Labs., Clark Univ., Worcester, Mass.* Several water soluble estrogen derivatives—estriol succinate, estrone carboxymethoxime, estradiol succinate and estradiol phthalate were tested with potato and mushroom tyrosinases, mushroom laccase and cytochrome oxidase at pH 7.2 in Barcroft-Warburg respirometers. With potato tyrosinase all these substrates show rapid oxygen uptake of equal rates but varying induction periods. The end products are reddish in color, have no estrogenic action and seem to be quinones. The oxidation is inhibited by cyanide, salicylaldoxime and other copper inhibitors. Stilboestrol is similarly oxidized. None of these substrates show significant oxygen uptake with any mushroom tyrosinase. With mushroom laccase rapid oxidation sets in but is quickly retarded or terminated. With potato tyrosinase about 3–4 atoms of oxygen per molecule of substrate are absorbed, while with laccase only one atom of oxygen. The laccase products are not colored but are biologically inactive. No action by cytochrome oxidase has been observed.

Pancreatic diabetes in the monkey. ISABELLE GRAYMAN (by invitation), NORTON NELSON (by invitation), SAMUEL ELGART (by invitation) and I. ARTHUR MIRSKY. *May Inst. for Medical Research, Jewish Hospital, Cincinnati, O.* Depancreatized Rhesus monkeys, maintained with insulin and a mixed diet adequate for growth, respond to the deprivation of insulin and food with a rapid development of ketonemia and ketonuria, so that in from 2 to 4 days the blood acetone bodies reach levels of from 170 to 225 mgm. per cent. Associated with the ketonemia is a moderate hyperglycemia. The animals become dehydrated, lethargic and subsequently comatose, and manifest Kussmaul breathing as well as cold

moist extremities. This syndrome is associated with a marked fall in the CO_2 content of the blood.

Insulin deprivation alone produces a slowly rising ketonuria and ketonemia, which reaches a maximum in about two weeks. This is associated with a marked loss in body weight. While the animal continues to eat, the CO_2 content of the blood is depressed only slightly, while a marked hyperglycemia exists. The syndrome of dehydration and subsequent coma does not occur. However, animals deprived of food following a period of insulin deprivation rapidly developed the syndrome of diabetic coma.

A glass membrane optical manometer. HAROLD D. GREEN. *Dept. of Physiology, Medical School, Western Reserve Univ., Cleveland, O.* (Demonstration.) Glass membranes are made by slightly drawing out 5 mm. pyrex thin walled tubing, sealing off and blowing a small rounded end. This is carefully heated at the end and a small bulb blown. The end of this bulb is again heated and blown producing an elongated thin-walled bulb. When sufficient thinness has been obtained in the end of the bulb a quick passage through the edge of the flame will produce a flattened membrane at the end of the bulb which should be from 3 to 6 mm. in diameter. A glass peg about ½ mm. in diameter and ½ mm. long is cemented to the outer edge of the membrane with water glass and the mirror mounted on this peg with hard wax. Annealing in an oven hardens the water glass joint.

The glass membranes are mounted on the manometers with conical "syringe-type" joints to facilitate obtaining a horizontal swing of the beam.

These membranes have been found to have a higher combination of frequency and sensitivity than the glass spoon manometers or the rubber membrane manometers. Their glass construction facilitates detection and removal of air bubbles. (*Supported by a grant from The Commonwealth Fund.*)

Modification of blood flow in skin and muscle of dogs in hemorrhagic shock. HAROLD D. GREEN and (by invitation) RICHARD S. COSBY and ROBERT N. LEWIS, *Dept. of Physiology, School of Medicine, Western Reserve Univ., Cleveland, O.* Many of the phenomena of shock may be due to a reduction of blood volume. To study the influence of this factor on the regional blood flow in shock we recorded *a*, the arterial inflow into organs temporarily perfused *in situ* with heparinized blood; *b*, peripheral venous pressure; *c*, aortic pressure; *d*, hematocrits, and *e*, blood viscosity. Artifacts due to collateral circulation were avoided by the technique demonstrated at this meeting by Green, Cosby and Radzow.

No significant difference has been discovered *a*, in the changes of blood flow; *b*, in the vascular reactions, or *c*, in the venous pressure fluctuations during temporary reduction of blood volume by hemorrhage compared with those occurring during shock spontaneously developing during an experiment or induced by a prolonged interval of low blood pressure produced by hemorrhage with subsequent reinfusion. Under chloralozane anesthesia blood flow decreased before arterial pressure, and flow almost ceased when aortic mean pressure reached 60 to 80 mm. Hg. The changes in hematocrit and viscosity were insufficient to greatly affect flow during the control period and, since peripheral venous pressures fell rather than rose, the vascular lumen must have decreased. This was due in part to augmented sympathetic discharge since nerve section or death caused in some experiments a return of the flows toward control rates. In others presence of some chemical agent or development of tissue edema may have operated. Under similar experimental conditions in barbital anesthetized dogs increase of muscle artery lumen often occurred. [*Supported by a grant from The Commonwealth Fund.*]

Evaluation of the collateral circulation artifacts which complicate studies of regional vascular reactions. HAROLD D. GREEN and (by invitation) RICHARD S. COSBY and KARL H. RADZOW. *Dept. of Physiology, School of Medicine, Western Reserve Univ., Cleveland, O.* (Demonstration.) Estimations of vascular reactions in perfused organs *in situ* are complicated, among other things, by the variable precapillary collateral communications. As a result, arterial inflow differs in an unpredictable way from the flow through the perfused vascular bed. In part, this variation depends upon the difference between aortic and perfusion pressures and upon the vasomotor tone of the collateral communications.

Perfusion with heparinized blood at the existing aortic mean pressure minimizes this collateral exchange. However, the rate of flow through the perfused vascular bed is not a linear function of perfusion pressure but requires determination of a control pressure flow curve. This curve is constructed by 1, measuring the rate of arterial inflow at each of a series of constant pressures from the zero-flow pressure to well above aortic pressure; 2, plotting the simultaneous flow and pressure values, which lie along a line which may be straight or concave to the flow axis; 3, again determining the zero-flow pressure (which will be much lower) after temporarily clamping all the collateral arteries, and 4, drawing a second line of similar shape through the aortic pressure point of the first line and through the zero-flow pressure point with the collateral arteries clamped. This

last line gives the approximately correct relation between perfusion pressure and flow through the perfused vascular bed.

In skin, muscle, intestine and brain, inflow at pressures 50 mm. Hg above or below aortic pressure may differ as much as 50 per cent from the flow through the vascular bed. This difference is often reduced to zero during hemorrhagic shock. [*Supported by a grant from The Commonwealth Fund.*]

The magnitude of the effective collateral circulation following sudden arterial occlusion. HAROLD D. GREEN, ROBERT N. LEWIS (by invitation) and KARL H. RADZOW (by invitation). *Dept. of Physiology, Medical School, Western Reserve Univ., Cleveland, O.* (Read by title.) The viability of the tissue supplied by an artery is, following sudden occlusion of that artery, dependent upon the magnitude of the blood flow from collateral arteries which reaches the vascular bed of the occluded artery by way of the precapillary collateral communications. This is often roughly estimated by severing the artery and measuring the rate of backflow from the distal end.

In order to determine the actual collateral flow we record the pressure in the artery by way of a side branch, at the moment of and following occlusion proximal to the pressure connection. This peripheral arterial pressure falls rapidly following occlusion from aortic pressure to a stable pressure around 50 to 70 mm. Hg in muscle, skin, intestine and brain and to around 10 to 20 mm. Hg in the coronary arteries. We then plot the relation of perfusion pressure to flow through the vascular bed by the method demonstrated at this meeting by Green, Cosby and Radzow. The rate of flow on this plot at the pressure recorded in the distal end of the artery, after pressure equilibrium is attained following occlusion, is the rate of flow from the collateral arteries through the vascular bed of the occluded artery.

In all regions except the heart backflow from the cut end of an artery equaled about $\frac{1}{3}$ to $\frac{1}{2}$ the inflow at aortic pressure, whereas the effective collateral circulation was usually about one-half this, i.e., around $\frac{1}{5}$ to $\frac{1}{4}$ the normal inflow from the aorta. The effective collateral circulation was less than 1/70 the normal inflow in the heart. [*Supported by a grant from the Commonwealth Fund.*]

Forty-eight-hour response to androsterone in the immature male rat. R. R. GREENE and M. W. BURRILL (by invitation). *Dept. of Physiology and Pharmacology, Northwestern Univ. Medical School, Chicago.* (Read by title.) The authors have demonstrated that 24 hours after the administration of testosterone propionate to male rats 32–38 days old, there is an increase in seminal vesicle weight. The responses showed a good linear relationship to the logarithm of the dose, but animal variability was high. Subsequently it was demonstrated that the response 48 hours after treatment was similar, but less variable in the 20–22 day-old rat.

Androsterone has been administered to 146 rats, 20–22 days old, and the weights of the seminal vesicles determined 48 hours later. With 20 or more animals to a group the mean responses were also a linear function of the logarithm of the dose. A significant increase in seminal vesicle weight 10.3 per cent) was obtained with the smallest dose used (0.25 mgm.). The maximal dose (6.0 mgm.) caused an increase of 82.0 per cent. Half of the determinations for each dosage group were made in June, July and August; the remaining half in October, November and December. It was found that the mean responses for each dose were higher in the summer months than in the fall and winter months. The dose response curves, however, were parallel.

The value of a 48-hour test, such as this, for an assay procedure is limited by the low magnitude of response, the inherent animal variability and the seasonal variability.

The action of lactogenic hormone on tissues other than the mammary gland. R. O. GREEP, BACON F. CHOW and H. B. VAN DYKE. *Division of Pharmacology, The Squibb Inst. for Medical Research.* The experiments of Evans and colleagues who demonstrated that lactogenic hormone is the pituitary hormone maintaining corpus luteum function in rats have been confirmed in this laboratory and elsewhere. Other potentially important extramammary effects of the hormone, particularly on the gonads, have consequently been reexamined in rats.

Highly purified lactogenic hormone (20 I.U. per mgm.) was furnished by Dr. E. Schwenk. Its potency was independently verified by the systemic crop-gland test.

Lactogenic hormone caused no growth in hypophysectomized rats (total dose: 90 I.U.).

Immature female rats, one week after hypophysectomy, received 30 I.U. daily for 7 days. No interstitial cell-repair, follicular growth, or uterine enlargement could be detected.

In immature male rats hypophysectomized 3 days before and then treated with 30 I.U. daily for 7 days, the anterior prostate weighed slightly more than control prostates although atrophy was extensive and no testicular interstitial cell-stimulation was evident; therefore, a trace of metakentrin (ICSH) possibly was present. There was no thyroid stimulation. Immediately after hypophysectomy, adult males received 30 I.U. daily for 10 days. There was no effect on interstitial cells, accessory sexual organs or seminiferous

epithelium; partial adrenal maintenance was found.

None of the tissues mentioned in the foregoing descriptions was altered by injection of lactogenic hormone into normal immature males.

Augmentation of right coronary inflow during pulmonary artery stenosis. D. E. GREGG, W. H. PRITCHARD (by invitation) and R. E. SHIPLEY (by invitation). *Dept. of Medicine, Western Reserve Univ., Cleveland.* Phasic and mean right coronary inflows have been determined in anesthetized dogs with optically recording orifice meters and rotameters respectively.

Gradual elevation of right ventricular pressure (up to 80 to 90 mm. Hg) by clamping the pulmonary artery increases right coronary inflow considerably, despite a fall in aortic blood pressure. If the aortic blood pressure is maintained approximately at the control level, right coronary inflow increases further (up to 100 per cent) and remains at this level during elevation of right ventricular pressure. Upon release of the pulmonary clamp the inflow slowly returns to the control value. This flow increase exists throughout both systole and diastole (orifice plate meter).

At the height of the flow increase during pulmonary stenosis, the addition of coronary sinus occlusion may be without effect on right coronary inflow, or may reduce it slightly. Sinus occlusion alone causes either no change in right coronary inflow or a slight reduction.

In the left coronary artery the inflow is but little affected by pulmonary artery stenosis, but is reduced considerably by occlusion of the coronary sinus.

The operative mechanism for this flow increase in the right coronary artery is being investigated. [*Supported by a grant from the Commonwealth Fund.*]

Some errors in the application of the thermostromuhr method to the measurement of blood flow as shown by the rotameter. D. E. GREGG, R. E. SHIPLEY (by invitation), W. H. PRITCHARD (by invitation), R. W. ECKSTEIN (by invitation), J. DINGLE KENT (by invitation) and J. T. WEARN. *Dept. of Medicine, Western Reserve Univ., School of Medicine, Cleveland, O.* (Demonstration.) In the use of the direct current thermostromuhr for quantitation of blood flows in normal and anesthetized dogs, several factors other than rate of flow, may influence the empirical flow values obtained. The rotameter (and orifice plate meter) will be used to demonstrate the presence of some of these errors and their approximate magnitude. [*Supported by a grant from the Commonwealth Fund.*]

The effect of sodium sulfadiazine, sulfadiazine and sulfaguanidine on blood sugar and liver glycogen. ESTHER M. GREISHEIMER, ROBERTA HAFKESBRING and GRACE E. WERTENBERGER (by invitation). *Dept. of Physiology, Woman's Medical College of Pennsylvania, Philadelphia.* The method used was described in previous communications. The following values for blood sugar and liver glycogen were found:

	BLOOD SUGAR	LIVER GLYCOGEN
	mgm./100 cc.	*per cent*
Fasting	80	<0.018
Controls (glucose, no drug)	96	0.836
Fasting and sodium sulfadiazine (10%)	66	0.022
Glucose and sodium sulfadiazine (10%)	117	1.305
Glucose and sodium sulfadiazine (7.5%)	99	1.86
Glucose and sulfadiazine (1%)	118	1.65
Glucose and sulfaguanidine (1%)	89	1.388
Glucose and sulfaguanidine (7.5%)	107	1.36
Glucose and sulfaguanidine (10%)	100	1.825

These drugs do not seem to have the harmful effect on liver that was found with sodium sulfapyridine. They resemble sodium sulfathiazole in their action. [*Supported by a grant from the Committee on Therapeutic Research, Council of Pharmacy and Chemistry, American Medical Association.*]

Further studies on the preparation of kidney extracts effective in reducing the blood pressure in experimental hypertension. ARTHUR GROLLMAN, T. R. HARRISON (by invitation) and J. R. WILLIAMS, JR. (by invitation). *Dept. of Internal Medicine, Bowman Gray School of Medicine, Wake Forest College, Winston-Salem, N. C.* The renal extract previously described has been subjected to further purification. Because of the technical difficulties involved in filtration of acid aqueous extracts of fresh tissue, the kidneys (frozen pig kidneys obtained from the abattoir) have been dehydrated with alcohol and dried preliminary to their extraction. The resulting powder was extracted with acidulated water and subjected to an ammonium sulfate precipitation, as described previously (J. Biol. Chem. **134**: 115, 1940). (The crude ammonium sulfate precipitate has been prepared and supplied to us through the courtesy of Dr. George B. Walden and the Eli Lilly Company, to whom we are indebted for their coöperation.)

We have found that the principle effective in lowering the blood pressure in hypertensive animals is dialysable. It is also ultrafiltrable through collodion membranes prepared as described elsewhere (J. Gen. Physiol. **9**: 813, 1926). On dialyzing ammonium sulfate precipitate through viscing membranes, proteins as well as many other inert materials are retained, while the active principle passes through the membrane. The aqueous extract thus obtained is further concentrated by utilizing the relative insolubility of the active

principle in organic liquids (alcohol, acetone, ether), its behavior towards adsorbents such as charcoal and the formation of insoluble compounds in the presence of certain precipitants. Details of these procedures will be described elsewhere.

In order to avoid the justifiable criticism that an observed lowering of blood pressure in the hypertensive animal may be due to the injection of nonspecific, toxic impurities present in renal extracts, we have avoided parenteral administration in all our assays. Our most concentrated extracts, when administered orally in relatively small doses, are still capable of reducing the blood pressure of rats rendered hypertensive by various procedures. [*Aided by grants from the John and Mary R. Markle Foundation and from Mr. Joe Werthan.*]

Activity in the dorsal spino-cerebellar tract and its effect in the cerebellum. HARRY GRUNDFEST and BERRY CAMPBELL (by invitation). *Labs. of the Rockefeller Inst. for Medical Research, New York City.* Electrical activity is initiated in the dorsal spino-cerebellar (Flechsig's) tract of the cat by afferent stimulation of the tibial or the peroneal nerve or of nerves to the muscles of the hind limb, but not by stimulation of the saphenous nerve or of contralateral nerves. The tract begins as low as the fourth lumbar segment and extends into the vermis of the cerebellum where the fibers are distributed bilaterally to the lobulus centralis and to the rostral part of the culmen.

A maximal afferent stimulus causes a volley of activity in Flechsig's tract which lasts up to 10 milliseconds. The early part of the volley arises from stimulation of Clarke's cells by collaterals from the primary neurons, the cells responding after a synaptic delay of 0.5 to 0.9 msec. Collaterals from more than one primary neuron may stimulate one cell. Reexcitation of the nucleus by internuncial chains of various length contributes to the later tract activity. Clarke's cells respond to repetitive synaptic stimulation at a frequency above 1,000/second. The responses of the tract fibers, as recorded with micro-electrodes, are spikes lasting 0.5 msec.

Impulses ascend in Flechsig's tract with velocities up to 160 mps. and arrive at the medulla 1 to 2 milliseconds ahead of those which ascend in the fasciculus gracilis. The first electrical activity in the cerebellum is that of impulses afferent in Flechsig's tract. These impulses alone initiate considerable cerebellar activity but additional activity is produced by impulses arriving later via other pathways.

Thresholds of ventricular fibrillation by galvanic currents. Effects of barbiturates and of citrate. ALBERTO GUEVARA ROJAS (by invitation), A. SIDNEY HARRIS and DEMETRIO SODI PALLARES (by invitation). *Dept. of Physiology, Western Reserve Univ. School of Medicine, Cleveland, O.* Measurement of the direct current (milliamperes; duration up to 5 sec.) required to induce fibrillation in ventricles of cats and dogs when the anode is on the ventricle and the cathode planted subcutaneously has been found to be a sensitive index of the susceptibility of the ventricle to fibrillation. Both electrodes are chlorided silver wires. In determinations the current is reversed in alternate trials, i.e., the stigmatic pole is cathode and then anode, but with equal current applications about 96 per cent of the fibrillations are of anodal origin.

Controls which must be observed follow: 1, the stigmatic electrode should be jacketed, and the contact kept free of collected fluid; 2, the stigmatic electrode may be moved only within a limited area all parts of which have been found experimentally to have equal sensitivities; 3, anesthesia (barbital Na or dial) should be calculated for a minimal surgical depth. If determinations are begun within the second hour after administration of the anesthetic, fibrillation threshold values may show a declining trend. At the end of about two hours the readings level off, showing spontaneous variations of approximately 0.2 ma. about an average threshold of 2.5 to 4.0 ma. With a smaller contact the value is less.

Results. After the steady state is reached additional dial elevates the threshold in a smooth curve. Barbital produces an oscillating threshold with the minimum at the control level. Citrate temporarily lowers the threshold. Decerebrate animals show threshold elevation after dial, but do not survive many fibrillations. [*This research was supported by a grant from the John and Mary R. Markle Foundation.*]

A spectroscopic method for determination of carbon monoxide in air. F. G. HALL. *Duke Univ., Durham, N. C.* A sample of air containing carbon monoxide is introduced into a tonometer and equilibrated with a dilute solution of hemoglobin. The concentration of carbon monoxide is determined with a photo-electric-spectrophotometer. a formula is given for the calculation of carbon monoxide percentage in the air sample. The chief advantage of this method is the rapidity with which determinations can be made.

Rôle of a renal humoral mechanism in shock. ANGIE S. HAMILTON (by invitation) and DEAN A. COLLINS. *Dept. of Physiology, Temple Univ., School of Medicine, Philadelphia, Pa.* Since hemorrhage evokes a renal humoral mechanism (Hamilton and Collins, 1941), it may be involved in shock.

After blood pressure had been maintained by hemorrhage at shock levels for about 1¾ hours, nephrectomized dogs gave a slightly increased average response to injected renin (kindly

supplied by Dr. I. H. Page), while intact animals showed absent or feeble reactions (12 dogs anesthetized with chloralose). These findings suggest that the kidneys may liberate enough renin to cause tachyphylaxis and thus disable their own compensatory mechanism. Under similar conditions, pressor activities of arterial and renal venous blood were almost equal. With less severe hypotension and evident compensation tachyphylaxis to renin was not observed.

The blood pressures of 3 intact and 3 adrenalectomized dogs were lowered to shock levels (60–30 mm Hg) by histamine. Pressor activity, demonstrated by injection into a nephrectomized dog, consistently appeared. Renal origin was indicated by arteriovenous differences and by disappearance of pressor activity in arterial blood following nephrectomy in 2 adrenalectomized animals.

Control bloods of 3 dogs, acutely adrenalectomized before technique was perfected, gave profound falls of blood pressure in nephrectomized dogs. Other dogs, showing normal pressures and no depressor material following adrenalectomy, exhibited blood pressures at shock levels and pressor activity in the blood after small hemorrhages (e.g., 19 cc. per kgm.) or small histamine dosage (e.g., 0.147 mgm. per kgm.). This extreme sensitivity was evident as early as 2¼ hours following adrenalectomy. [*Aided by a grant from the American Philosophical Society*].

The recoil curves of the circulation in three dimensions. W. F. Hamilton and Philip Dow. *Univ. of Georgia School of Medicine, Augusta.* To record simultaneously in three dimensions the recoil forces from blood movements in the body has involved the construction of an apparatus in which forces applied in each dimension can be recorded independently of those applied in the other directions. The apparatus which was finally developed for this purpose will be described and some of the difficulties met with during its evolution will be gone into.

The curves are different in the three dimensions and it is hoped that their analysis will offer some reconciliation of the varied viewpoints in regard to the ballistocardiogram.

Curves will be presented from the human, under various physiological conditions, and from "cardio aortic" models in which known variations in stroke volume, and in the time relations of the ejection curve can be independently made.

Particular attention will be paid to the phenomena which occur at the very beginning of ejection in the hope of unraveling the puzzle as to why the first strong down-stroke in the human ballistocardiogram does not occur as early in the cardiac cycle as would be expected on *a priori* grounds and from experience with models.

Physiological effects of artificially administered acetylcholine and eserine. E. T. Hansen (by invitation), J. J. Worzniak (by invitation) and Robert Gesell. *Dept. of Physiology, Univ. of Michigan.* Effects of acetylcholine and eserine were re-studied to further our knowledge of the neurohumoral concepts of Loewi, Dale and Cannon in the light of the newly proposed acid-neurohumoral mechanism. (Mich. Hosp. Bull. **7:** 94, 105, 1941.) The variable modifications of breathing (central action) observed by others is confirmed. But of greater significance is our more common result of highly coordinated hyperpnea in which both inspiratory and expiratory components were increased. This coordinated increase of breathing produced at both center and chemoceptor is definitely potentiated by eserine. The intensity of response to acetylcholine varied with the site of injection. Vertebral excelled carotid injections, due probably to greater distribution of acetylcholine to bulbar centers. The results on injections of lactic acid appear comparable to those of acetylcholine. Such common effects of acetylcholine and acid suggest a common mechanism of stimulation —injection of acetylcholine increasing the neurohumoral stimulant directly and acid increasing it indirectly by retarding the destruction of physiologically deposited acetylcholine at the synapses. Some evidence suggested that acid plus acetylcholine is more effective than acetylcholine alone. Eserine produced prolonged hyperpneas and undoubted subnormal cH levels during which excessive artificial hyperventilation had little effect on breathing. Anticholinesterase activity possibly interfered with the usual rapid destruction of acetylcholine even in an excessively alkaline media. Every incoming signal (acetylcholine deposition) is thus potentiated and the respiratory act is driven with supernormal intensity.

The rôle of the liver in alimentary azotemia. Henry N. Harkins, Brock Brush, R. T. Boals and C. Frank Chunn (introduced by L. R. Dragstedt). *Dept. of Surgery, Henry Ford Hospital, Detroit.* By alimentary azotemia is meant the increase of blood urea nitrogen which follows the introduction of protein, either that ingested as food, or occurring as a result of hemorrhage, into the upper intestinal tract of man or animals. It is well known that the liver is of major importance in urea formation. In agreement with this is the clinical observation of Stadie and Van Slyke that in cases of acute yellow atrophy of the liver, low blood urea values occur.

In our experiments, whole blood or red blood cells were given to prepared dogs by stomach tube in an amount that would produce an appreciable degree of alimentary azotemia in normal animals. The prepared dogs were of three types: Eck fistula, reverse Eck fistula and hepatectomized animals. In the animals with Eck fistulas there was practically no rise of blood urea nitrogen, in the reverse Eck fistula dogs the increase was ac-

tually greater than in an average animal with intact portal circulation, while in the liverless animals the blood urea nitrogen level fell or remained constant despite the administration of blood by duodenostomy tube.

In the hepatectomized animals, prothrombin readings were taken at the same time as the blood urea readings. The fall in blood prothrombin somewhat paralleled the decrease in blood urea nitrogen. These experiments indicate the importance of the rôle of the liver and portal circulation in urea metabolism especially that type of urea formation which results from the intragastric administration of blood or blood cells and which we have termed alimentary azotemia.

Fibrillation and other functional changes in mammalian ventricles resulting from coronary occlusion. A. SIDNEY HARRIS, ALBERTO GUEVARA ROJAS (by invitation) and DEMETRIO SODI PALLARES (by invitation). *Dept. of Physiology, Western Reserve Univ. Medical School, Cleveland, O.* Recent papers from our laboratory have shown that fibrillation induced by a single brief stimulus or by galvanic current is introduced by an accelerating series of discharges originating at the stimulated point. Evidence that the discharges produce the conditions that permit fibrillation was presented.

Electrograms recorded from three places on the ventricle with riding contiguous electrodes simultaneously with an electrocardiogram have been used to study events leading to the fibrillation that occurs in dog and monkey ventricles when the anterior descending ramus of the left coronary artery is occluded. These fibrillations, also, are introduced by accelerating trains of idioventricular systoles. When local beats and fibrillation failed to appear within about ten minutes after occlusion, fibrillation usually would occur soon after removing the clamp, readmitting blood to the ischemic area. An accelerating series of local discharges precede fibrillation as with other techniques.

Exploration of the ventricle by varying the placements of leads during different occlusions revealed evidence of the development of relatively great potential differences, both R spikes and monophasicities, in the transition zone between the ischemic and non-ischemic areas. This zone appears the likely source of idioventricular excitations. Measurement of precedences of local spikes from many points indicate that beats may originate at a number of different loci about this border, but there appears to be a predominating point. This one assumes command during the group leading into fibrillation.

In the ischemic area the R spikes widen and occur progressively later with respect to the pre-R deflection and electrocardiogram. [*This research was supported by a grant from the John and Mary R. Markle Foundation.*]

Some differences between urogastrone and enterogastrone. S. C. HARRIS (by invitation) and J. S. GRAY. *Dept. of Physiology and Pharmacology, Northwestern Univ. Medical School, Chicago.* (Read by title.) The fact that both enterogastrone, obtained from intestinal mucosa, and urogastrone, obtained from urine, inhibit gastric secretion and motility has suggested that the two substances may be identical. However, it has been found that procedures which influence the production of enterogastrone do not similarly influence the output of urogastrone (Am. J. Physiol. **134**: 623, 1941). In view of these differences a comparison has been made between certain chemical and physiological properties of enterogastrone and urogastrone extracts. In regard to chemical properties it has been found that urogastrone differs from enterogastrone in that the former is *a*, stable to peptic digestion; *b*, soluble in methyl alcohol, and *c*, more stable to chemical manipulation. In regard to physiological effects, it has been found that urogastrone and enterogastrone differ in regard to their inhibitory potencies on gastric secretion and motility. These findings support the interpretation that enterogastrone and urogastrone are separate entities. [*Aided by a grant of the Committee on Endocrinology of the National Research Council.*]

The action of monocrotaline, isatidine, pterophine, sceleratine, and the alkaloid of senecio carthamoides. PAUL N. HARRIS (by invitation), ROBERT C. ANDERSON (by invitation) and K. K. CHEN. *Lilly Research Labs., Indianapolis, Ind.* Monocrotaline is an alkaloid present in the seeds of *Crotalaria spectabilis* and *C. retusa*. Isatidine occurs in *Senecio isatideus* and *S. retrorsus*, pterophine in *S. pterophorus* and *S. ilicifolius*, and scleratine in *S. sceleratus*. The alkaloid of *Senecio carthamoides* melts at 220–221°C. and has a probable formula of $C_{18}H_{25}O_5N$, but has not been named by Professor Roger Adams.

By intravenous injection in mice of appropriate doses of the above substances, dissolved in dilute hydrochloric acid, death slowly resulted. The median lethal doses based on fatality within one week are as follows:

ALKALOID	MEDIAN LETHAL DOSE ± STANDARD ERROR
	mgm. per kgm.
Monocrotaline	261.3 ± 12.53
Isatidine	834.5 ± 50.25
Pterophine	58.13 ± 3.73
Sceleratine	139.40 ± 9.33
Alkaloid of *S. carthamoides*	68.32 ± 2.44

Necroscopy revealed a resemblance of the pathological changes caused by these alkaloids. The

liver was invariably effected. With monocrotaline, isatidine, sceleratine, and the alkaloid of *S. carthamoides*, the majority of the animals developed central necrosis of the liver with congestion, but with pterophine the lesion was predominantly periportal. Ascites, hydrothorax, and pulmonary edema were frequently present. Additional experiments in rats by intravenous injection of a single dose of monocrotaline gave rise to similar results.

The distribution of lactic acid between the blood and aqueous humor and the rate of glycolysis of the crystalline lens. WILLIAM M. HART (introduced by H. M. Hines). *Dept. of Ophthalmology, State Univ. of Iowa, Iowa City.* The concentration of lactic acid in the aqueous humor has been determined variously by different investigators with published values ranging from 24 mgm. per cent for the rabbit to 205 mgm. per cent for the horse. The present study employs the sensitive micromethod of Barker and Summerson (J. Biol. Chem. **138**: 535, 1941). All animals were fasted at least 24 hours.

The lactic acid content of the blood of the fasted rabbit ranged from 54 to 123 mgm. per cent (leg vein). The lactic acid content of the aqueous humor of the same animals ranged from 63 to 108 mgm. per cent. In cattle the blood level ranged from 48 to 54 mgm. per cent and the aqueous humor of the same animals 37 to 48 mgm. per cent of lactic acid.

The lens is known to metabolize sugar. Accordingly, the right eyes of newly weaned rabbits were rendered aphakic and the animals permitted to grow to maturity. The author is thus far unable to confirm the observation of F. P. Fischer (Ergebn. de Physiol. **31**: 533, 1941) that the aphakic eye of the rabbit contains less lactic acid than the normal eye.

Data will be presented on the rate of glycolysis of the intact lens in vitro. [*Aided by The John and Mary R. Markle Foundation.*]

A method of recording the dimensions of muscle fibre striations during contraction. E. NEWTON HARVEY and F. J. M. SICHEL. *Physiological Labs., Princeton Univ. and Univ. of Vermont Medical College.* A single muscle fibre is mounted isometrically on a cover slip, which is then supported above a slide fastened to the vertical stage of a microscope (tube horizontal). Long thin platinum wire electrodes serve to stimulate the fibre in Ringer's solution. A 15 ampere carbon arc illuminates the fibre, whose high power (62X objective and 20X ocular) image is concentrated by a cylindrical lens on a slit, so that the muscle striations are parallel to the direction of movement of a supersensitive film directly behind the slit, in a recording camera. With a 0.5 mm. slit and film moving 500 mm. per second, a photograph of the striations in one plane is obtained, equivalent to taking 1,000 pictures a second. The stimulus is recorded by the flash of a mercury glow lamp which illuminates the slit. As the muscle contracts, any change in the dimensions of the striations can be immediately detected as well as the amount of the isometric contraction. Lantern slides of the records will be shown.

The unit of fibrillary activity and the site of origin of fibrillary contractions in denervated striated muscle. GEORGE J. HAYES (by invitation) and CLINTON N. WOOLSEY. *Dept. of Surgery and Physiology, Johns Hopkins Univ., School of Medicine, Baltimore, Md.* Whether the unit of fibrillary activity in denervated striated muscle is the entire muscle fiber or a fraction thereof is unsettled. A particularly favorable structure in which to study this question is the unilaterally denervated diaphragm of the rat.

Visual observation shows that the whole fiber is involved. This can be demonstrated under the microscope in the living anesthetized animal on opening the abdomen or after death when only a few fibers are still active.

With electrical methods we have found that potential changes do not originate at random throughout the muscle as would be expected if fibrillation were limited to parts of muscle fibers. Rather, fibrillation potentials take origin from a very specific place—the end-plate zone, at the centers of the muscle fibers—and spread thence toward both ends. In spite of asynchronous activity in various units it is easy to demonstrate that the contractions originate at the end-plate zone even in vigorously fibrillating muscle.

Further evidence for the site of origin of the fibrillary contraction has been secured by excising the end-plate zone from actively fibrillating muscle. Fibrillation continues in the strip of muscle bearing the end-plate zone but ceases in the other sections as soon as they are cut off.

It is not clear whether the rhythmic discharges originate in the muscle fibers themselves or in the remaining elements of the degenerated end-plates. [*Sponsored by the National Foundation for Infantile Paralysis.*]

The chloride content of isolated muscle fibers. L. V. HEILBRUNN and PAULINE G. HAMILTON (by invitation. *Dept. of Zoology, Univ. of Pennsylvania, Philadelphia.* Fibers of the frog adductor magnus muscle were dissected out in chloride-free nitrate Ringer's fluid. They were washed twice in this fluid, each fiber being washed separately, and the chloride content of approximately 70 fibers was then determined. Numerous such tests were made. The volume of the fibers used in a given test was determined by the direct measurement of each fiber, and the weight was calculated from the volume. In order to determine the chlo-

ride analytically the fibers were frozen with dry ice and were then extracted either with hot water or with concentrated nitric acid. Wigglesworth's micro method was used for the analysis. The chloride values obtained from the hot water extracts were higher than those obtained from the nitric acid extracts. This was true both in the case of the isolated fibers and also for pieces of whole muscle.

The concentration of chloride found in the fibers was almost as high as that found for pieces of whole muscle. Thus, in one series of 16 experiments, the average chloride concentration of the fibers was 80 per cent of that found for whole muscle. Moreover, histochemical tests showed the presence of chloride within isolated muscle fibers. These results are opposed to the usual view that muscle fibers contain no chloride.

The influence of ageing on the cardiovascular response to exercise. F. A. HELLEBRANDT and PERCY M. DAWSON. *Dept. of Physiology, Univ. of Wisconsin Medical School, Madison.* One method of studying the effect of ageing upon fitness for muscular work is to observe the same subject over a span of years. This study is concerned with the influence of severe exercise on one of us (P.M.D.). The earliest observations were made by Eyster in 1914. Similar experiments on the bicycle ergometer were performed in 1926, 1927 and 1929. The present series consisted of seven exercise periods carried to the limit of effort.

The subject has a long exercise history. His work achievement at the end of the seventh decade was about 72 per cent of his performance 27 years earlier and approximately 80 per cent of that displayed at the age of 53. His optimum speed of pedalling had dropped 12 per cent. The maximum heart rate, systolic pressure and pulse pressure attained during exercise fell short of the peak values of 1914. Recovery from the acute effects of the effort was prompt and uneventful. Transitory abnormalities in cardiac rhythm occurred once. A mild post-exercise negative phase in blood pressure followed the severest ride. There was less resilience than earlier extending the number of rest days required between rides. Staying power improved about 150 per cent during the brief period of training.

Although maximum strength and endurance are reached at about 30 and the ability to carry on sustained work is thought to dwindle during the latter years of mature life, our 68-year-old subject demonstrated surprisingly little decline in the ability to do hard muscular work and to recover therefrom.

The influence of the regulation army pack on body alignment and postural stability. F. A. HELLEBRANDT, E. CORINNE FRIES (by invitation) and ELEANOR M. LARSEN (by invitation). *Dept. of Physiology, Univ. of Wisconsin Medical School, Madison.* (Static demonstration.) The subjects of the experiment were seven army officers of different build ranging in body weight from 62.1 to 96.9 kilos and in stature from 170.6 to 191.4 cm. Serial biplane posture photographs were taken at five second intervals for two minutes and synchronized with simultaneous biplane center of gravity observations. Each subject was observed five times, twice without and three times with a regulation army pack. No accessory equipment was carried. The pack was adjusted in regulation position, placed too high or too low. The demonstration will shown the influence of the pack on the symmetry and stability of the stance and upon the magnitude of the gravitational stresses acting on the joints of the supporting limbs.

The response of the blood pressure and pulse rate of the new born rat to changes in body temperature. HENRY F. HELMHOLZ, JR. (introduced by F. C. Mann). *Division of Experimental Medicine, Mayo Foundation, Rochester, Minn.* Since the new born rat has been shown to be essentially poikilothermic, studies on the blood pressure and pulse rate in relation to changes in the temperature of the body were undertaken. The carotid artery of new born rats was canulated and the mean blood pressure and pulse rate were recorded optically using a damped glass spoon manometer. Rectal temperatures were recorded simultaneously by means of a differential thermocouple. The temperature of the animals was influenced by means of water flowing through a lead platform on which the rats were placed. At body temperatures of 37°C. the blood pressure varied between 20 and 30 mm. Hg and the pulse rate varied between 150 and 200 per minute. Lowering the body temperature from 5 to 10°C. caused a decrease of both blood pressure and pulse rate of from 30 to 50 per cent. Elevation of body temperature caused increases of blood pressure of 10 to 30 per cent and increases in pulse rate of from 50 to 100 per cent.

Vascular changes associated with short periods of raised intrapulmonary pressures. J. F. HERRICK, C. F. CODE, A. R. MACLEAN (by invitation) and F. C. MANN. *Division of Experimental Medicine, Mayo Foundation, Rochester, Minn.* The aim of this investigation was to study the circulatory changes in the dog under conditions as similar as possible to those which pertain in the human during the Flack (Respiratory) Endurance or Fatigue Test. This test has been employed as an index of physical fitness particularly in the examination of prospective aviators.

All observations were made on dogs anesthetized with pentobarbital sodium. The blood flow was determined with the thermostromuhr, and the venous and arterial pressures were measured with the glass spoon manometer.

A cannula, placed in the trachea, was connected to a T-tube, one limb of which permitted normal respiration, the other afforded connection to a reservoir of positive pressure of any desired magnitude. Blood flow measurements were made in the thoracic inferior and superior vena cavae and the carotid artery in the neck. The pressures in the jugular vein and carotid artery were recorded.

Positive pulmonary pressures of 30, 40 and 60 mm. Hg delivered through the tracheal cannula for periods of one minute caused a prompt and marked reduction of the blood flow in all the vessels studied. The systemic arterial pressure fell immediately but, when the positive pressures were not too large, a partial return to the control level took place. The pressure in the jugular vein rose rapidly, the magnitude of the rise depending upon the degree of intrapulmonary pressure.

The mechanism of the azotemia of experimental intestinal obstruction. RAYMOND C. HERRIN. *Dept. of Physiology, Univ. of Wisconsin Medical School, Madison.* Theoretically two factors might be responsible. There might be impairment in the renal excretion of urea. McQuarrie and Whipple have reported renal impairment in intestinal obstruction in dogs and Gamble and McIver reported that some rabbits with obstruction were anuric. In a few rabbits studied in the summer of 1939, anuria was present but in this series of rabbits none were anuric. The urea excretion during the period of intestinal obstruction for 6 rabbits varied from 19 to 68 per cent of the normal. In control experiments ether anesthesia and surgical manipulation of the intestine resulted in a marked reduction in urea excretion but never as marked as occurred in obstruction. Another factor likely responsible for this impairment is the chemical changes of the blood.

A second possible factor responsible for the elevation of blood urea might be an increased rate of urea production. By determining the total body water and serum urea it was possible to calculate the total urea content of rabbits in the normal and near death in obstruction. These data, with the urinary urea made possible a determination of the rate of urea production in the animal. In 4 or 6 rabbits, urea production was slightly less or more than the normal. In 2 rabbits urea production, during obstruction was about twice the normal. However, these 2 had very low normal rates of urea production and in obstruction the rate approximated the normal of other rabbits.

Effect of iodoacetate on survival period during hypoxia. HAROLD E. HIMWICH, HERMAN HERRLICH (by invitation), EDITH RICH (by invitation), ROBERT BARSTOW (by invitation) and JOSEPH F. FAZEKAS (by invitation). *Dept. of Physiology and Pharmacology, Albany Medical College, Union Univ., Albany, N. Y.* In the present experiments the role of glycolysis in the maintenance of cerebral function has been studied during hypoxia. Adult dogs were anesthetized with Dial. The effects of hypoxia (8.5 per cent oxygen) and the injection of Iodoacetate (32-40 mgm. per kgm.) on the cerebral arteriovenous oxygen difference and survival time were determined separately and together.

Dogs tolerate either 8.5 per cent oxygen or survive the injection of Iodoacetate in the carotid arteries for more than one hour. During hypoxia the cerebral arterio-venous difference becomes reduced while with Iodoacetate it becomes slightly greater though blood glycolysis is inhibited as much as 70 per cent. Together Iodoacetate and hypoxia result in respiratory failure in 2 to 6 minutes.

Experiments with Iodoacetate on animals respiring air demonstrate *in vivo* the maintenance of a normal cerebral arterio-venous oxygen difference despite a great inhibition of blood glycolysis. The energy produced by cerebral glycolysis does not permit indefinite survival wnen the oxygen concentration is reduced to 8.5 per cent but it is adequate to support cerebral metabolism for periods longer than one hour. If the supply of anaerobic energy is inhibited by the injection of Iodoacetate while breathing 8.5 per cent oxygen, the animal succumbs rapidly.

Single fiber analysis of inhibition of inspiration. ROBERT HODES and M. G. LARRABEE. *Johnson Foundation, Univ. of Pennsylvania, Philadelphia.* Inhibition of inspiratory motoneurones by brief trains of afferent impulses was studied in anesthetized cats (Dial). The superior laryngeal nerve was stimulated electrically while recording the impulses in single efferent fibers of the phrenic nerve.

A brief burst of stimuli during inspiration delays the next impulse by an interval which maybe as great as six times normal, following which the frequency returns to the control value after one or two impulses. The duration of the pause increases progressively with the number of stimuli until enough are applied to stop inspiration completely. Occasionally two or three impulses are discharged after the pause before inspiration finally stops. The subsequent expiratory pause is usually of approximately normal length.

A suitable number of stimuli, which at the start of inspiration causes a brief pause produces progressively shorter pauses as inspiration proceeds, until, after a critical time the inspiratory discharge is stopped. Progressively later application of the stimuli after this critical time causes progressively increasing expiratory pauses.

The central action of a brief burst of afferent impulses may persist for over 0.2 sec., as shown by a reduction of the number of stimuli required

to stop inspiration. The above effects are observed after section of the vagi.

The number of impulses required to curtail inspiration is increased during hyperpnea produced by inhalation of high carbon dioxide or low oxygen, but is not increased by low oxygen if the carotid sinus and vagus nerves are sectioned.

The conduction of cortical impulses and the motor management of convulsive seizures. PAUL F. A. HOEFER (introduced by F. A. Mettler) and J. LAWRENCE POOL (introduced by F. A. Mettler). *Dept. of Neurology, Columbia Univ. College of Physicians and Surgeons, and the Neurological Inst. of New York.* "Slow" impulse groups originating (under barbiturate anesthesia) in the motor cortex are conducted in the corresponding pyramidal tracts and in the corpus callosum. At rest they do not reach or at least do not fire the opposite cortex or the lower motor neurone. In deep ether anesthesia no clear-cut correlation of cortical activity and that of descending tracts is found, while under light ether and in curarized animals bursts of "spike" impulses originating in the cortex could be identified in various parts of the brainstem as well as in the pyramidal tracts. Convulsive seizures produced by electrical stimulation were conducted chiefly in the pyramidal tracts and in structures tentatively identified as being in the medial reticular substance. Bursts of activity synchronous with seizure activity were also found in the region of the dorsal column nuclei and the secondary sensory pathways. By section of one or both pyramids or section of the entire corpus callosum (anatomical controls) the clinical pattern of the seizures was widely modified and the thresholds for generalized seizures were markedly raised. Tonic and clonic seizures were, however, obtained even with both pyramids sectioned. Under full ether the electrocorticogram is abolished and the seizure threshold is about six times normal. Seizure potentials however are of higher amplitude. With complete curarization seizure potentials are obtained without visible movement. Both slow (10/20/sec) and spike activity is found in conducting brainstem structures during seizures. A three-channel inkwriter and a three-channel cathode-ray oscillograph were used. The experiments were made on cats.

Further studies in the metabolism of progesterone. M. M. HOFFMAN (by invitation) and J. S. L. BROWNE. *McGill Univ. Clinic, Royal Victoria Hospital, Montreal, Canada.* The metabolism of progesterone was studied in normal and castrated male rabbits. The steroid, dissolved in sesame oil, was given subcutaneously or orally. Urine was collected quantitatively during the administration period and for the succeeding 5 days. The urine adjusted to pH 1, was heated in the autoclave at 121° for 30 minutes and then extracted with ether. The ether-soluble material, washed free of acids and phenols, was divided into alcoholic and non-alcoholic fractions. From the former, crystalline pregnane—3 (α), 20 (α)—diol, identified by mixed melting determination and by preparation of derivatives, was isolated by chromatographic analysis.

The male rabbit, as does the human male, converts progesterone into pregnanediol. The extent of this conversion approximates 10 per cent in both normal and castrated animals. The failure of the absence of the testis to affect this conversion is interesting in view of the fact that Marker et al. (J. Am. Chem. Soc., **60**: 2931, 1938) isolated pregnanediol from bull urine but not from the urine of steers (J. Am. Chem. Soc. **61**: 1287, 1939). That the urinary pregnanediol is conjugated as in human urine was shown by the isolation of sodium pregnanediol glucuronide (81.3 mgm.) by the Venning method (J. Biol. Chem. **119**: 473, 1937) from the urine of rabbits receiving progesterone (550 mgm.) orally.

The role of the faeces in the excretion of pregnanediol is now being investigated. Preliminary results indicate that only animals receiving progesterone by mouth excrete pregnanediol in the faeces (yield—7.1 per cent). (Aided by a grant from the Banting Research Foundation.)

Sweat concentration after desoxycorticosterone injection. C. G. HOLLAND (by invitation), R. F. KLINE (by invitation) and S. W. BRITTON. *Physiological Laby., Univ. of Virginia Medical School, University.* (Read by title.) Recognition that desoxycorticosterone exerts a profound influence on renal secretion and conserves electrolytes in the body suggested the desirability of testing its action on sweat secretion. Five human subjects were used.

Exposures were made in an electrical dry-air cabinet for periods ranging from 30–90 minutes, the usual time limit being 60 minutes. Generally two or more preliminary control runs without treatment were made, followed by exposures while under desoxycorticosterone influence; in some cases controls were made between or after hormone tests. Three days or more were allowed as rest periods between runs. The sweat cabinet temperature was usually kept under 110°F., and the oral temperature of the subjects usually varied between 99° and 100°F.

Sweat collections were made from the same areas of the trunk at approximately 10-minute intervals. Two or three urine samples were taken before, and one or two after exposure in the cabinet; one blood sample was drawn before and one after the test.

Sodium, chloride and urea determinations were made on all samples. Ten or 20 mg. of desoxycor-

ticosterone were given in two doses within two hours of exposure.

Under the short-term conditions involved, the blood and urine changes observed were not significant. Moderate reductions in sweat sodium, chloride and urea were apparent after desoxycorticosterone injection, however; usually, these averaged between 15 and 20% below normal control values. It is indicated therefore that desoxycorticosterone acts similarly on the sweat and renal organs.

The effect of positive and negative intra-thoracic pressure on cardiac output in the dog. J. P. HOLT (introduced by Hampden Lawson). *Dept. of Physiology, Univ. of Louisville School of Medicine, Louisville, Ky.* Cardiac output was measured by the direct Fick method in seven barbitalized dogs in the supine position. Blood samples were taken from the right auricle and the femoral artery. Oxygen consumption was measured by means of a spirometer using atmospheric pressure, positive pressure of 16 centimeters of water, and negative pressure of 16 centimeters of water in the spirometer.

The average cardiac output when oxygen under a negative pressure was breathed was 13 per cent greater than the average output when oxygen at atmospheric pressure was breathed. Under positive pressure the average output was 17 per cent ess than the control output.

In an earlier report (Am. J. Physiol. **134:** 292, 1941) it was shown that when air under a negative pressure was breathed right auricular pressure decreased but peripheral venous pressure remained constant. In nine experiments when air under a negative pressure of 16 centimeters of water was breathed, the average difference between right auricular and peripheral venous pressure was 194 per cent greater than the average difference when atmospheric pressure was used. Since breathing air under a negative pressure of 16 centimeters of water increased the cardiac output only 13 per cent above the control output, it would appear that the maintenance of a high peripheral venous pressure, when auricular pressure is decreased, is not the result of an increased cardiac output; but is the result of an increase in the resistance to the flow of blood from the periphery to the right auricle caused by the veins becoming partially collapsed just before entering the chest.

Altered responses to various respiratory modifiers as a result of brain stem transections. JUAY HONG (by invitation) and HAYDEN C. NICHOLSON. *Dept. of Physiology, Univ. of Michigan.* In anesthetized dogs it was found that incomplete midpontine section might transform the hyperpnea of saphenous nerve stimulation into respiratory slowing. Similarly, the respiratory acceleration sometimes produced in lightly anesthetized dogs by pulmonary inflation might be replaced by pure slowing following partial pontine transection.

In decerebrate dogs whose respiration following mid-pontine transection and vagotomy was characterized, as described in the paper of Nicholson and Hong, by rhythmically repeated periods of maintained inspiration, the duration of these periods was greatly prolonged by intravenous injection of sodium cyanide. Before the period of prolonged inspiration terminated there occurred an increase in the amplitude of the superimposed respirations associated with a decrease in the depth of the maintained inspiration. Both saphenous stimulation and central vagal stimulation prematurely terminated the period of maintained inspiration, its termination being preceded by evidences of inspiratory stimulation. Continued vagal stimulation prolonged the subsequent expiration. Intermittent central vagal stimulation might cause rhythmic breathing, normal in type, at a frequency corresponding to that of the stimulus.

Following complete transection through the upper medulla both central vagal stimulation and cyanide injection were without effect upon respiration, while saphenous stimulation might still cause hyperpnea.

The effect of a protein-deficient diet upon the serum phosphatase and hepatic dye clearance of dogs. VICTOR H. HOUGH (by invitation) and SMITH FREEMAN. *Dept. of Physiology and Pharmacology, Northwestern Univ. Medical School, Chicago.* Protein has been removed from the diet of 31 dogs during 43 experimental periods. In every case protein depletion has resulted in a significant rise in the serum phosphatase activity which increase varied from 10 Bodansky units in an adult dog to 177 units in a puppy. The usual increase in most adult dogs was 20–30 units above the normal level of 2–4 units per 100 cc. of serum. In approximately one-third of the depletion experiments the liver function was followed at 14-day intervals by means of the Rose Bengal dye clearance test, and there was found to be a parallelism between the increase in serum phosphatase and the decrease in the rate of dye clearance. Dogs maintained on a protein-deficient diet for as little as 14 days usually show a definite reduction in the ability to remove this dye.

Egg white, casein and beef hearts, when added to the protein-deficient diet restored the blood values to the normal range. Choline, added to the deficient diet, retarded the rate of alteration of liver function, or when fed to the depleted animal after impaired liver function existed, restored the dye clearance to normal and lowered the serum phosphatase almost to the normal range. Cystine appears to intensify the manifestations of protein

deficiency. The effect of methionine is under investigation.

The activity of the descending duodenum in man during nausea produced by caloric stimulation of the semicircular canals. F. J. INGELFINGER and R. E. Moss (introduced by F. H. Pratt). *Evans Memorial, Mass. Memorial Hospitals, and Dept. of Medicine, Boston Univ. School of Medicine.* When a balloon is inflated in the proximal portion of the descending duodenum in man, it will move backwards into the stomach; if inflated in the distal portion, it will pass into the transverse duodenum and jejunum. Because of these antagonistic actions, a tandem two-balloon system can be "anchored" in the descending duodenum, if one balloon is inflated in the proximal, the other in the distal portion. A continuous record of the duodenal activity can then be taken.

A series of such records was taken on human subjects before, during, and after nausea produced by caloric stimulation of the semicircular canals. This method of producing nausea avoids the confusing side-reactions of emetic drugs. With the onset of nausea, a striking diminution in the caliber of the descending duodenum usually occurred. In some instances, this was followed by the expulsion of both balloons into the stomach. Similar records were obtained during nausea which followed the subcutaneous injection of morphine sulfate. No reverse peristalsis was observed.

It would appear that nausea, whether produced by stimulation of the semicircular canals or by the administration of morphine, usually as accompanied by a generalized contraction of the descending duodenum which tends to expel the duodenal contents through a patent pylorus into the stomach.

Preservation of human hemoglobin solutions without methemoglobin formation. JOYE E. JACOBS (by invitation), ALAN HISEY (by invitation) and WILLIAM R. AMBERSON. *Dept. of Physiology, School of Medicine, Univ. of Maryland, Baltimore, and Dept. of Biochemistry, George Washington Univ. School of Medicine, Washington.* Human hemoglobin solutions made isotonic with blood plasma are sterilized by Seitz filtration. The solutions are completely reduced by vacuum extraction with a Hyvac pump. They are then sealed in vacuo in glass ampoules. Although exposed to light and warm room temperatures these solutions show no methemoglobin formation for periods up to three months.

Direct measurements on the influence of caffeine, bromides and sodium amytal in man. EDMUND JACOBSON. *Laby. for Clinical Physiology, Chicago.* Measurements of neuromuscular states in man secured with sufficiently sensitive electrical apparatus can now be reported following the administration of caffeine sodium benzoate, triple bromides and sodium amytal.

Action-potentials were measured over half hour test periods under controlled conditions employing the Integrating Myovoltmeter. Tests in various orders were made on different days in fifteen healthy subjects. Ninety grams of triple bromide were given by mouth in divided dosage, two hours and one hour before measurement on three different days. Control tests without medicine were run on additional days. As a second set of control tests, sterile water was injected intramuscularly on three other days. Intramuscular injections were made also of sodium amytal (1⅞ gr.) and of caffeine sodium benzoate (7½ gr.). So far as possible, the subjects were kept in ignorance of the agents employed and of the purposes of test.

The composite graph for all the subjects shows lower neuromuscular action-potentials following the bromide administration, as compared with control tests, but the reduction is not extreme. Accordingly, since the dosage employed was six times as great as standard, it seems probable that the effects of this sedative in standard dosage as commonly prescribed are partly due to the knowledge of the patient that he has taken a sedative.

A more marked sedative effect was noted following sodium amytal intramuscularly. On the other hand, action-potentials were distinctly increased in the group following caffeine intramuscularly. Individual curves often fail to follow the trends shown in the corresponding composite graphs.

Further observations on the dynamics of fluid flow in an elastic system. K. JOCHIM (introduced by L. N. Katz). *Cardiovascular Dept., Michael Reese Hospital, Chicago.* Using an improved artificial circulation model, we have previously reported that if other factors are kept constant, increasing the peripheral resistance may cause the pulse pressure to increase, decrease, or remain unchanged, depending on the slope of the pressure-volume curve of the elastic system used. We have found that the P-V curve of an elastic system obtaining under dynamic conditions when the volume is constantly changing, differs from that determined under static conditions. In the former, the pressure at a given volume is higher than in the latter. The discrepancy becomes greater the more rapidly the system changes in volume. This may be explained by the increased force necessary to overcome the "plasticity" of the elastic material as it is made to change rapidly in size.

Pulse volume was found to remain unchanged as peripheral resistance was increased, regardless of the type of elastic system used. Furthermore, no redistribution of flow between systole and diastole occurred, as determined by the electro-

magnetic flowmeter. The ratio of systolic to diastolic flow remained constant in any one experiment. It can be shown that the ratios of systolic to diastolic areas under the pressure curves are not an index to flow ratios in a system where turbulent flow obtains and velocity is not directly proportional to pressure.

These effects of changing peripheral resistance on pulse pressure and pulse volume are the same regardless of the rate of the pump or stroke volume, and hence they are qualitatively independent of minute-volume flow. [*Aided by the Amer. Assn. Adv. Sci.*]

Immediate effects of tobacco smoke on the dog's respiration and blood pressure. JOHN F. JOHNSON (by invitation) and CLAUDE V. WINDER. *Dept. of Physiology, Univ. of Michigan.* In each of 14 dogs lightly anesthetized with evipal, "mouth smoke" from branded or rolled ciagarettes, pipe or cigar has been repeatedly admitted into the inspiratory side of valves connected with the trachea. In intact animals, light tobacco smoke caused the simple, abrupt, transient increase in respiration characteristic of carotid body excitation. More dense smoke initiated the "carotid body" effect or cut into the first of it with an abrupt, very transient reduction in amplitude, usually accompanied by acceleration, or a "tense" apnea. Persisting signs of "lightness" commonly followed the excitation. In certain animals the initial reduction in amplitude persisted so the subsequent excitation was characterized mainly by acceleration.

Vagotomy eliminated the initial reduction in amplitude, leaving a typical, sometimes monstrous, abrupt, carotid body-type of stimulation, predominantly in amplitude. Cutting the carotid nerves in addition eliminated the typical respiratory excitation. Tobacco smoke then caused a more or less marked depression in amplitude, with rate increasing or decreasing slightly, or remaining the same. With the carotid nerves cut and the vagi intact, the initial depression of amplitude with acceleration preceded a more mild (aortic body?) stimulation of breathing or merged into a more extended accelerated, restricted respiration.

There was usually more or less transient hypertension associated(?) with the early reflex respiratory effects. It tended to be more marked after cutting the carotid nerves. A commonly more gradual, prolonged hypertension persisted after both sets of nerves were cut. Coffee and tea smoke were essentially without effect.

An attempt to correlate bleeding-time with capillary fragility in man. F. T. JUNG, JOHN M. BRADY (by invitation) and HENRY V. UPHOLT, JR. (by invitation). *Dept. of Physiology and Pharmacology, Northwestern Univ. Medical School, Chicago.* (Read by title.) It is conceivable that varying susceptibility of the capillaries to rupture might be a factor in the wide variations of bleeding-time found in normal subjects. With this in mind, a number of medical students were tested, each being subjected to both tests on any given day. Bleeding-time was measured by the Ivy-Duke's method; capillary fragility was scored according to the number of petechiae produced by applying a measured suction to an area of skin. Both tests were repeated several days later on each subject. Despite the homogeneity of the group, wide variations were found from person to person and from day to day. This wide range would have tended to accentuate, rather than conceal, a correlation between the two variables. Nevertheless, the correlation between the bleeding-time and the capillary fragility in a given individual at a given session proved to be so low that extension of the study to a larger group of normal subjects was not undertaken.

Energy expenditure in swimming. PETER V. KARPOVICH and NATHAN MILLMAN (introduced by W. B. Cannon). *Dept. of Physiology, Springfield College, Springfield, Mass.* Twenty-four men and women were tested. Breath was held during swimming. The amount of energy used was calculated from the oxygen debt. Douglas-Haldane method was used. Swimming strokes studied were: crawl, back (inverted crawl), breast, butterfly and overarm side.

From the standpoint of cost for *good* swimmers the strokes may be arranged in the order of an increasing cost as follows: for speeds of 2 ft/sec—crawl, back, breast, side and butterfly; at 2.5 ft/sec—butterfly becomes more economical than side; and at 3 ft/sec—butterfly becomes more economical than breast.

The strokes may be divided into two major groups: crawl (crawl and back) and breast (breast, butterfly and side). At a speed of 2ft/sec. the number of large calories spent per minute in various strokes is respectively: 9, 10, 11, 12, 14. The energy spent in the crawl group is approximately proportionate to the square of the speed. This is not true for the breast stroke group.

There is a great fluctuation in the amount of energy spent by different swimmers for one and the same stroke, depending upon the degree of proficiency. This fluctuation is greater for lower speeds (difference may reach 367%). For the higher speeds (4–5 ft/sec. fluctuations are less, because in order to develop that speed, the subject should possess a high degree of efficiency. [*Aided by a grant from the Elizabeth Thompson Science Fund.*]

The effect of desoxycorticosterone acetate in shock due to massive venous thrombosis of a leg. An experimental study.: L. N. KATZ and (by invitation) S. KILLIAN, S. PERLOW and R. ASHER.

Cardiovascular Dept., Michael Reese Hospital, Chicago. Recently we described a method for producing shock in dogs. This consisted of occluding the iliac vein in one limb and injecting a suspension of lampblack in physiological saline below the occlusion. Associated with this induced state of shock and eventual death was the tremendous edema of the occluded limb. In our control series of 15 dogs to date, 12 died in shock in from 3½ to 13½ hours, while the other 3 survived.

Using this method we studied the effect of desoxycorticosterone acetate (doca) on this type of shock. One series of 11 dogs were primed, i.e., received an average of 25 mgm. of doca, subcutaneously, 24 hours prior to the "shock procedure," and an average of 35 mgm. of doca during the following 24 hour period. Eight of the 11 animals thus treated survived; 2 died in shock in 41 and 29 hours, respectively; and 1 died with pulmonary edema in 12 hours. Thus 9 of the 11 dogs primed with doca lived longer than our control dogs or survived the shock procedure.

In another "unprimed" series, 3 dogs received an average of 40 mgm. of doca, subcutaneously, starting 2 hours after venous occlusion and continued over a period of 12 hours, while 6 dogs received only 10 mgm. of doca, ten minutes to 2½ hours prior to the occlusion and an average of 36 mgm. during the following 12 hours. In this series only 1 animal survived; 1 died of pneumonia 15 hours later, while the other 7 died of shock—5 in from 4½ to 12 hours, the other 2 at 15 and 20 hours, respectively.

Our results suggest that doca can serve as an effective prophylactic agent in the type of shock produced, provided it is given sufficiently ahead of the shock procedure; but its use after shock has started is equivocal. [*Aided by the A. D. Nast and Emil and Fanny Wedeles Funds for Cardiovascular Research.*]

Distribution in leads I and III of potentials applied to the surface of the heart. W. KAUFMAN (Fellow of the Dazian Foundation) (by invitation), L. H. NAHUM and H. E. HOFF. Direct currents were applied to various surfaces of the heart in dogs through circular tin electrodes 1 cm. in diameter and the resultant deflections were recorded in the standard leads. When electrodes were placed near the centers of the anterior surface of the right ventricle and the posterior surface of the left ventricle, the maximum deflection was found in lead III while little or no deflection appeared in lead I. Negativity of the electrode in the right ventricle gave an upward deflection while negativity at the left ventricle produced a downward deflection. Application of current through electrodes at the centers of the anterior left ventricle and the posterior right ventricle gave a maximum deflection in lead I and little or no deflection in lead III. Negativity at the right ventricle produced at upward deflection while negativity at the left ventricle yielded a downward deflection.

When a thin rod was passed through the heart joining the centers of the anterior right ventricle and the posterior left ventricle, the projection of the axis so formed on the horizontal plane of the body was roughly parallel to lead III. Similarly, the projection of the axis joining the center of the anterior left ventricle and the posterior right ventricle was roughly parallel to lead I.

These experiments confirm previous observations on the nature of leads I and III and afford a physical explanation for them. [*Supported by a grant from the Fluid Research Fund, Yale University School of Medicine.*]

The influence of bile on gastric secretion. JERZY KAULBERSZ and JAMES M. WINFIELD (introduced by Charles G. Johnston). *Dept. of Surgery, Wayne Univ. College of Medicine.* Dogs were prepared with Heidenhain pouches, Pavloff pouches and gastric fistulae.

When 2 grams of bile were placed into fasting dogs' stomachs, 5 to 35 minutes before histamine injection, diminution of both secretion and acidity was noted in the Heidenhain pouches in 50% of the experiments. When bile was given after histamine injection no inhibitory effect on secretion occurred nor was there any increase in the amount or acidity of the secretion in the pouch.

Bile introduced into dogs stomachs with Pavloff pouches induced an increased amount of gastric secretion from the pouches after histamine injection.

In gastric fistulae dogs, the amount and acidity of gastric secretion was found to be exceedingly high in several instances following histamine injection and introduction of bile. These high readings were never equalled by the histamine effect alone. In certain instances histamine and bile produced exceedingly small amounts of secretion.

It would seem that the bile effect on gastric secretion is partially stimulatory and partially inhibitory. Atropine injection does not influence the inhibition but does nullify the stimulation of secretion. In Heidenhain pouches the inhibitory effect is predominant, while in Pavloff pouches the stimulatory effect is most marked. In gastric fistulae dogs the combined inhibitory and stimulatory effects produce a variety of results. These results seem to justify the supposition that bile introduced into the stomach initiates a nervous stimulation producing an increase of gastric secretory activity and that when the bile progresses into the intestine, absorption produces a decrease of gastric secretory activity.

Toxicity of paraldehyde for guinea pigs. F. A.

KAY (by invitation), EMMETT B. CARMICHAEL and G. W. PHILLIPS (by invitation). *Bryce Hospital, Tuscaloosa, and Dept. of Physiological Chemistry, School of Medicine, Univ. of Alabama, University.* (Read by title.) Normal adult guinea pigs, that weighed from 400 grams to 963 grams, were used in this series of experiments. There were 32 females and 73 males. Each of the females had delivered one or more litters. The drug was diluted with nine volumes of water and administered intraperitoneally. The dose of this solution varied by increments of 0.25 cc. from 12 cc. to 14 cc./kilo. The minimum lethal dose of this 1:10 solution of paraldehyde was 12.5 cc./kilo and the average lethal dose was about 13 cc./kilo.

Pancreatectomy incompatible to life in the presence of near-total hypophysectomy in the dog. A. D. KELLER, J. M. BRUHN, C. F. BARTON (by invitation) and W. E. LAWRENCE (by invitation). *Dept. of Physiology and Pharmacology, Univ. of Alabama, University.* For this study the same animals described in the abstract by Bruhn, Keller, Lawrence and Barton were utilized. The pancreas was removed from 3 to 8 months after the hypophysectomy procedures were executed.

There is no alleviation of diabetes mellitus following pancreatectomy until more than 90 per cent of the distal portions of the pars anterior and posterior lobe are removed.

Following an *ordinary hypophysectomy* pancreatectomy results in a maximal Houssay effect (the major part of hypophysial stalk tissue remains). These animals exhibit no glycosuria under any conditions nor a fasting hyperglycemia. The glucose tolerance curve is raised and prolonged. The life of the animal is greatly prolonged beyond that for otherwise normal pancreatectomized dogs but loss of weight is progressive and death occurs when emaciation becomes extreme.

In *near-total hypophysectomized* dogs (only proximal remnants of stalk tissue remain) *pancreatectomy results in death in from 4 to 8 hours.* These animals recover from the ether and appear normal for from 2 to 4 hours when progressively sluggishness, muscular weakness, prostration and finally death supervene. The period of prostration is associated with polypneic respiration and periodic straining movements. Death is preceded by a characteristic period of gasping. This sequence of events is not altered by 1, maintenance of blood sugar at or above the normal level, or 2, administration of adrenal cortical extract (Upjohn's), desoxycorticosterone (Ciba) or anterior lobe extract (Parke-Davis "total extract"). The successful fortification with anterior lobe extract being attested to by the return of insulin tolerance and basal metabolism to near the normal.

Sulfonamide action on pneumococcus and staphylococcus at various oxygen concentrations and in the absence of oxygen, determined by measuring manometrically the bacterial metabolism. WALTER KEMPNER. *Dept. of Medicine, Duke Univ., Durham, N. C.* Oxygen consumption and anaerobic CO_2 formation of cultures of varying composition of pneumococcus Type I, II, III, and of four strains of hemolytic staphylococcus were measured by the Warburg technique at 38°C. in air and at 7.5, 4, and 0 volume per cent oxygen. The sulfonamide concentrations were 0.001 to 0.00001 molar.

The sulfonamides did not affect either oxygen consumption or anaerobic fermentation of cultures that did not grow. The effect of the various sulfonamides on growing cultures was found to be *independent* of the oxygen concentration. The order of effectiveness of the drugs tested proved to be identical in air, at lowered oxygen tension and in complete anaerobiosis. With pneumococcus the least effective drug was sulfanilamide, next was sulfadiazine; then sulfapyridine, disulon, and and sulfaquanidine, of about equal potency; sulfathiazole was the most effective. With staphylococcus the order was: sulfanilamide, sulfaquanidine, sulfapyridine, disulon, sulfadiazine, sulfathiazol, for all oxygen concentrations.

The fact that the inhibitory action of the sulfonamides on pneumococcus and staphylococcus growth is unaltered, whether the bacteria gain their energy by oxidation or by anaerobic fermentation, shows, just as does the anaerobic p-aminobenzoic acid effect on sulfonamide-pneumococcus cultures, that the sulfonamides do not act by way of inhibiting bacterial oxidation or fermentation, nor by way of inhibiting the removal of bacteriostatic products specific for either metabolism.

Influence of lowered barometric pressure on the E. E. G. under various conditions. M. KESSLER (by invitation), H. HAILMAN (by invitation) and E. GELLHORN. *Depts. of Physiology and Psychiatry, Univ. of Illinois College of Medicine, Chicago.* Unanesthetized rats provided with phonograph needles as electrodes (Hoagland) were subjected to lowered barometric pressure. In 5 minutes a pressure of 200 mm. Hg was reached at which the rats were kept for 30 minutes (I), or for 5 minutes only and then exposed to 160, 140, 120 and 100 mm. Hg for equal periods (II). Rats subjected to (I) showed regularly spindles from the 15th to the 30th mm. which were usually preceded by a marked temporary depression of the potentials. In (II) the depression was followed by a gradual restoration of the E.E.G. in spite of further lowering of the barometric pressure. The E.E.G. remained normal until coinciding with gasping it was again greatly depressed (at 100 mm. Hg).

Fasted rats subjected to (I) showed diminished, delayed, or no spindles at 200 mm. Hg.

Benzedrine (5 mgm./kgm. subcutaneously) pre-

vents in all instances the occurrence of spindles at 200 mm. Hg. In contradistinction to the control group which is depressed the benzedrine rats are very active throughout the experiment. If, however, benzedrine rats are subjected to very low pressures (II) they are less resistant than control rats and gasping occurs earlier.

The experiments with benzedrine indicate that there need not be any correlation between the resistance of an animal to oxygen lack in very severe and rather moderate conditions. Procedures and drugs delaying death in severe anoxia may not be of any benefit to the organism under a less drastic anoxia.

The disappearance of the dye T-1824 from the blood stream. BARRY G. KING, ENID T. OPPENHEIMER and KENNETH S. COLE. *Dept. of Physiology, College of Physicians and Surgeons, Columbia Univ., New York City.* The course of the disappearance of the blue dye T-1824 from the blood stream of normal dogs was studied by spectrophotometric determinations of the dye concentration in successive serum samples. In six experiments the samples were withdrawn over a period of 24 hours after the injection. Although the time-concentration curves may be approximated by a straight line over short periods, they are not strictly linear and show that the dye disappears at a constantly diminishing rate.

The disappearance curves were analyzed in an attempt to discover the nature of the process by which the dye leaves the circulation. It was found that over long periods of time the best experimental fit was obtained when the dye was assumed to disappear as the square root of the time. This suggests that a diffusion process may be involved.

Work is now in progress to test the advisability of calculating the plasma volume by using the square root relationship to correct for the dye lost during the mixing period.

Anti-sulfanilamide activity of glycine, serine and allothreonine in E. coli. HENRY I. KOHN and JEROME S. HARRIS (by invitation). *Depts. of Physiology and Pharmacology, Pediatrics and Biochemistry, Duke Univ. School of Medicine, Durham, N. C.* Using a strain of coli which grows well in a basal medium of inorganic salts and glucose, it was found that of the amino acids only methionine showed appreciable antisulfonamide action. Since this was less than that of any of the usual commercial "peptones," it was obvious that other substances might exist which could enhance the action of methionine. We have therefore tested all of the commonly available amino acids by adding them individually to a medium composed of basal medium *plus* methionine. Under these conditions glycine, serine, and allothreonine are active; and also xanthine. The actions of methionine, glycine, serine and xanthine are additive, and together they are somewhat less active than 1 per cent proteose-peptone (Difco). Although precisely these relationships may not hold in other species, they illustrate a system which stands in contrast to *p*-aminobenzoic acid. The latter, for our strain of coli, is a perfect antagonist by itself, whereas the substances mentioned above show their full activity only in concert, and probably belong to the type which we have called "secondary" antagonists (J. Pharmacol. **73**: 383, 1941).

The secretion of phosphatase by the intestine of the dog. A. J. KOSMAN (by invitation), JERZY W. KAULBERSZ (by invitation) and SMITH FREEMAN. *Dept. of Physiology and Pharmacology, Northwestern Univ. Medical School, Chicago.* The secretion of intestinal phosphatase was determined in both acute and chronic experiments. A high phosphatase activity was found in the samplings after feeding the following substances to dogs with an enterostomy at three different levels: milk, casein, olive oil, beef heart, sucrose and prepared dog food. The greatest values were obtained with olive oil and casein. With olive oil, the average values in Bodansky units per 100 cc. of intestinal fluid were 2920 at the duodenum, 3118 at the jejunum and 17,785 at the terminal ileum; after casein feeding the values were 2170, 4404 and 23,229 at the duodenum, jejunum and terminal ileum respectively. Isolated loops of duodenum, jejunum and ileum were thoroughly washed with saline and their phosphatase secretion determined after continuous irrigation for several hours with isotonic saline, or after injection with eserine. In such experiments the phosphatase activity at the 3 levels was reversed, the duodenal fluid showing the greatest activity, the jejunum slightly less, and the ileum the least. The phosphatase activities of the uncontaminated secretions of the duodenum, jejunum and ileum obtained after eserine injection were 3150, 2735 and 436 Bodansky units per 100 cc. of secretion respectively. The colonic secretions of chronic colon fistula dogs were found to contain about 275 units of phosphatase per 100 cc. The duodenum and jejunum are the principal sources of phosphatase in the intestinal tract, the increase in phosphatase content of the intestinal contents being due apparently to concentration of the latter as they move caudalward.

Failure to produce arterial hypertension by intracisternal injection of kaolin. E. H. LAMBERT (by invitation) and G. E. WAKERLIN. *Dept. of Physiology, Univ. of Illinois College of Medicine.* (Read by title.) Attempts to produce a sustained arterial hypertension by intra-cisternal injection of kaolin have been uniformly negative in rats, rabbits and dogs, although the mode of preparation, technique of injection, and amount of kaolin suspension were varied. The symptomatology following

kaolin and the autopsy appearance of the central nervous system have not differed from those described by others. Comparison in dogs of the course of blood pressures concurrently taken by direct (mean) and indirect methods indicates that the positive results of others probably depend upon the use of the latter methods.

Charts for osmotic pressure of blood. Application of formulas for renal arteriolar resistance. HAROLD LAMPORT (introduced by Fred A. Mettler). *Dept. of Neurology, College of Physicians and Surgeons, Columbia Univ. and the Neurological Inst., New York City.* Formulas published in 1941 for afferent and efferent renal arteriolar resistance after measurement of blood pressure, hematocrit, serum protein, inulin and diodrast clearances did not distinguish between serum proteins of varying albumin to globulin ratios. A chart has been prepared, using an empirical relationship of Wies and Peters, so that serum protein of any A:G ratio can be converted into the osmotically equivalent concentration of serum protein of 2.20 A:G ratio. Another chart is then used to find the osmotic pressure of this equivalent blood protein concentration, both systemically and after glomerular hemoconcentration.

The formulas are applied to Corcoran and Page's data for angiotonin in man and dogs, renin in dogs, sympathectomy for human hypertension, and late toxemias of pregnancy.

The previously held conception that increase in filtration rate with decrease in renal blood flow is caused primarily by efferent arteriolar constriction is not always true. In an actual instance, the opposite was found.

Analysis of how afferent and efferent arteriolar resistance should change with blood pressure, if homeostasis (constant renal blood flow and glomerular filtration rate) is to be maintained, indicates that afferent resistance alone should vary, increasing or decreasing with blood pressure. This is the finding, at least qualitatively, in the low blood pressure during spinal anesthesia and in the high blood pressure cases here presented: the ratio of afferent to efferent arteriolar resistance varies roughly with the blood pressure.

Lack of glomerular osmotic equilibrium seems probably in severe late toxemia of pregnancy and acute nephritis.

The discrimination of color and form at levels of illumination below conscious awareness. CARNEY LANDIS and W. EDGAR VINACKE (by invitation). *Dept. of Psychology, Psychiatric Inst. and Hospital, New York City.* When visual stimuli are presented to the light-adapted eye at intensities of illumination which do not arouse consciousness and the observer is asked to guess the color or figure presented previous investigators have found that such guesses are better than chance and that their relative accuracy increases with an increase of illumination approaching the level of awareness. To obviate the possibility that the stimuli might be unnoticed rather than below awareness the experiment was repeated with the dark-adapted eye and by controlling the conditions by using a constant duration of stimulation, a system of signals rather than verbal communication, and ratings of report enabling the subject to state whether he was conscious of the stimulus and if conscious what certainty of correctness of judgment he felt. Eight stimulus conditions involving various relationships between color and form were investigated. From evidence obtained from fourteen observers the following conclusions were drawn: 1. Judgments of yellow stimuli below the momentary conscious threshold are more accurate than are judgments of red and green. 2. Changes in color, form, or color and form, did not affect or increase the accuracy of the judgments. 3. With an increase in intensity of illumination there was no regular increase in the accuracy of judgments reported below the level of consciousness. 4. Judgments of stimuli slightly below the form and color thresholds are more accurate than chance would allow.

The effect of exposure to low atmospheric pressure on the adrenal glands and on carbohydrate metabolism in the rat. L. L. LANGLEY (introduced by J. F. Fulton). *Laby. of Physiology, Yale Univ. School of Medicine, New Haven, Conn.* The studies of Evans (Am. J. Physiol. **110**: 273, 1934) on activation of the adrenal glands by exposure to low atmospheric pressure have been repeated, and essentially confirmed. The only deviation from his results which has been encountered lay in the failure of the blood sugar of fasted animals (24 hrs.) to rise upon exposure to low atmospheric pressure. Under these conditions, however, the liver glycogen was markedly augmented.

The question arose whether the adrenal activation occurred in response to a greater need for blood sugar under these conditions, or whether the carbohydrate effects developed secondarily from increased adrenal activity. If carbohydrate were utilized at a greater rate under low atmospheric pressure, one would expect the blood sugar to be lower in an adrenalectomized animal maintained on a constant dosage of hormone when exposed to low atmospheric pressure while fasting, than in a fasting animal on the same dosage at sea level. Adrenalectomized rats were maintained on one, three and five cc. of adrenal cortical hormone (Wilson) while fasting for twenty-four hours at 20,000 feet, and at sea-level. The blood sugar was not lower in the group exposed to low atmospheric pressure. If the animal was exposed to the low atmospheric pressure for several days to allow acclimatization before fasting, the liver glycogen

decreased, as compared with the marked augmentation that occurred when first exposed. It is concluded, therefore, that the adrenal glands are activated by exposure to low atmospheric pressure only during the period of acclimatization, and that the carbohydrate changes are merely an expression of this activation. [*Aided by a grant from the Josiah Macy, Jr., Foundation.*]

Studies on the fate of nicotine in the dog. P. S. Larson and H. B. Haag (by invitation). *Dept. of Pharmacology, Medical College of Virginia, Richmond.* In man and the dog we have found about ten per cent of administered nicotine excreted as such in the urine. The fate of the remaining ninety per cent is unknown.

Recently it has been twice suggested and once denied that trigonelline is an end product of nicotine metabolism in man.

In the experiments reported here, four dogs were brought into dietary equilibrium following which nicotine was administered subcutaneously in sufficient quantity to triple the daily excretion of trigonelline if this be an end product of nicotine metabolism. Urine was analyzed for nicotine; for nicotinic acid, nicotinuric acid and trigonelline by the method of Perlzweig and co-workers and for trigonelline and *n*-methyl pyridinium hydroxide by a modification of Kodicek and Wang's procedure.

About ten per cent of the nicotine appeared unchanged in the urine. No increase in the excretion of trigonelline or *n*-methyl pyridinium hydroxide was observed. The nicotinic acid fraction of the urine showed a four-fold increase, equivalent to about ten per cent of the metabolized nicotine calculated as nicotinic acid. The substance giving this reaction is not nicotine. It is not nicotinic acid since the trigonelline excretion was not increased. It is not nicotinuric acid since acid hydrolysis does not increase the size of the nicotinic acid fraction beyond that anticipated from the normal excretion of nicotinuric acid.

We conclude that in the dog, the pyrrolidine component of nicotine is probably not oxidized to less than a two carbon side chain. We plan to investigate other possible degradation products of nicotine.

The effect of phasic contractions of the intestine on intestinal blood-flow. Hampden Lawson. *Dept. of Physiology, Univ. of Louisville School of Medicine, Louisville, Ky.* The rate of arterial blood-flow into short loops of ileum or jejunum was recorded in barbitalized dogs by differential arterial manometry. Simultaneous records were made of volume changes in the loop as an index to intestinal muscular activity. When the loop was undistended, or when it was distended at pressures less than 10 cm. water, each phasic contraction of the intestine was accompanied by a synchronous phasic reduction in arterial flow. When the loop was inflated, although the amplitude of the recorded intestinal contractions was usually augmented, the amplitude of the phasic changes in blood-flow was invariably reduced. Usually at distention pressures between 10 and 30 cm. water even the strongest rhythmic gut contractions were unaccompanied by rhythmic changes in blood-flow. These data can be explained by assuming that in the distended intestine vascular by-passes are opened, and so divert a considerable portion of the flow from the muscular layers of the gut wall.

Studies on the glycogen metabolism of atrophic and regenerating muscle. B. Lazere (by invitation), J. D. Thomson (by invitation) and H. M. Hines. *Dept. of Physiology, State Univ. of Iowa, Iowa City.* Experiments were carried out on the gastrocnemius muscle of the adult albino rat. The glycogen concentration in muscle proved to be a sensitive indicator for the onset of changes in muscle proved to be a sensitive indicator for the onset of changes in muscle characteristic of atrophy and regeneration. Immobilization of muscles by cast, denervation and tenotomy resulted in a marked decrease in the concentration of muscle glycogen before an appreciable loss of muscle mass, contractile power and creatine concentration had occurred. Conversely, regeneration was characterized by a rapid and complete restoration of glycogen concentration before appreciable recovery of muscle mass, strength and creatine concentration.

At certain times during regeneration muscles previously subjected to denervation but not to tenotomy exhibited glycogen concentration values considerably above those found in normal control tissue.

That the low concentration of glycogen in denervated muscle was not the consequence of fibrillary activity is evidenced by the finding of equally low values in afibrillary atrophy resulting from either tonotomy or immobilization with casts. Furthermore, the decrease in muscle glycogen was not prevented by the administration of quinidine in doses found by others to abolish or greatly lessen fibrillary activity.

Denervated muscles from adrenalectomized rats maintained on adrenalin free cortical extract exhibited the same decrease in muscle glycogen as denervated muscles from control animals. This finding is opposed to the idea that the low glycogen levels are the result of an increased sensitivity of the denervated muscle to circulating adrenalin.

Detoxification of pregnanediol in the liver. C. P. Leblond (introduced by H. Selye). *Dept. of Anatomy, McGill Univ., Montreal, Canada.* It is now generally accepted that in man progesterone is excreted as sodium pregnanediol glucuronidate.

Since the activity of progesterone is increased after hepatectomy (Selye, 1940), it has been concluded that the liver detoxifies progesterone, possibly transforming it into pregnanediol. Hence an attempt was made to see if the liver could also detoxify pregnanediol itself.

For this purpose the anesthetic activity of pregnediol was compared in normal and in hepatectomized animals. Injection of 20 mgm. of pregnanediol produced no or only a slight anesthesia in young normal female rats. The same dose had a definite anesthetic effect in similar animals after a partial hepatectomy, removing 75 per cent of the liver. It was concluded, therefore, that pregnanediol is at least partly detoxified by the liver and is probably not the last link of the various compounds that may result from progesterone degradation in the rat.

Studies on the activity of the goitrous thyroid gland. ALLEN LEIN (introduced by George M. Curtis). *Ohio State Univ., Columbus.* Goitrous thyroid glands were studied immediately following thyroidectomy. After removal, each gland was divided into several representative portions, based upon gross appearance and later checked by histological examination. Samples of each portion were rapidly taken for determination of the rate of oxygen consumption in a Warburg apparatus. Parallel samples were used for the chemical determination of protein-linked iodine and non-protein iodine.

The rate of oxygen consumption of thyroids from individuals with toxic goiters ranges from 1.5 to 4 times that of normal thyroid tissue. Non-toxic goiters yield a Qo_2 which approximates that of normal thyroid tissue. The administration of quantities of potassium iodide to patients with toxic goiter induces involution of the thyroid but does not appreciably lower the rate of oxygen consumption of this tissue.

Protein linked iodine occurs in high concentration in non-toxic goiters and is very low in toxic goiters not treated with iodine. However, iodized toxic goiters are high both in protein and in non-protein iodine. Most of the goiters studied exhibited a mosaic of morphological forms, represented by the occurrence of nodules, islands of hyperplastic tissue, etc. These structural varieties possess distinctive iodine concentrations.

The data resulting from these determinations have been correlated with the concentration of protein-linked iodine in the blood and with the clinical history of the patient.

Factors influencing micturition volume in the rat. WESLEY E. LEONARD (by invitation) and CHANDLER MCC. BROOKS. *Dept. of Physiology, Johns Hopkins Medical School, Baltimore, Md.* (Read by title.) Evidence has been obtained suggesting a hormonal factor in the modification of bladder responses observed to occur during pregnancy and following parturition.

Micturition volumes of rats vary considerably but are fairly characteristic of the individual. Rats weighing approximately 200 gms. have an average micturition volume of 0.6 cc. When the same animals are anesthetized and their bladders filled artificially micturition does not occur until a volume of approximately 0.9 cc. is reached.

During pregnancy micturition volumes gradu-all—increase. The change is noticeable on the10th-12th day. Immediately following parturition there is an enormous (5 to 10 fold) increase in tolerated bladder size. The total daily urine output remains relatively constant. During the subsequent period of nursing, micturition volumes decrease but remain 3 to 5 times the normal volume. Following weaning the volume soon returns to normal. The death or removal of the young shortly after parturition results in an immediate return to normal micturition volumes.

Intramuscular injections of Progynon-B (200 R.U. daily for 7 to 14 days) are followed by a similar increase in micturition volume. Artificially filled bladders of anesthetized injected animals tolerate 2 to 5 times the normal volume before emptying. This change though generally beginning toward the end of the injection period frequently does not begin until 7 to 10 days after cessation of injections. Two to three weeks are required for recovery. *This work was aided by a grant (to Dr. Philip Bard) from the Committee for Research in Problems of Sex, National Research Council.*

Studies on the mechanism of insulin action in skeletal muscle in vitro. R. LEVINE (by invitation), R. N. FEINSTEIN (by invitation) and SAMUEL SOSKIN. *Dept. of Metabolism and Endocrinology, Michael Reese Hospital, and the Dept. of Physiology, Univ. of Chicago, Chicago, Ill.* We have previously confirmed Gemmill's original observation that insulin increases the rate of glycogen deposition from glucose, in rat diaphragm in vitro. In the present work it has been shown that various skeletal muscles of the rat give similar in vitro effects with insulin, and a step by step analysis of insulin action has been made on the basis of the generally accepted scheme for glycogenesis.

Our data indicate that insulin promotes the phosphorylation of glucose either directly or indirectly. If it is a *direct* influence, it is not concerned in a reaction catalyzed by an enzyme identical with the "hexokinase" isolated from yeast. We have found that in skeletal muscle in vitro: *a*, glycogenesis from glucose is increased in the presence of pyruvate; *b*, insulin increases CO_2 production from pyruvate; *c*, insulin increases the esterification of inorganic phosphate. From these

facts it seems likely that insulin promotes the phosphorylation of glucose *indirectly*, by increasing the formation of labile phosphate groups elsewhere in the scheme, probably below pyruvate. This agrees with our previous in vivo and in vitro evidence that insulin catalyzes the conversion of glucose into some intermediary (phosphorylated) metabolite which precedes and is necessary for both glycogen formation and sugar utilization. However the present work indicates that this is not the primary effect of insulin, and narrows the field of search for the basic reaction.

Frequency-localization in the auditory cortex of the monkey. J. C. R. Licklider and K. D. Kryter (introduced by E. A. Culler). *Laby. of Psychology, Univ. of Rochester, Rochester, N. Y.* Spatial representation of stimulus-frequency has been studied in the auditory cortex of the macaque monkey by an oscillographic method developed in previous experiments on the cat. Records obtained, in five monkeys, from various electrode placements within the auditory cortex show that eight representative frequencies from 125 to 8000 cycles activate the region differentially. The lower frequencies produce their maximal effects in the antero-lateral part of the region, the higher frequencies in the postero-medial. The frequencies are disposed in a sharper and more nearly linear gradient than was found in the cat, suggesting, in conjunction with other evidence, that spatial differentiation in the acoustic system is more fully developed in higher forms.

Activity in pathways concerned with long spinal reflexes. David P. C. Lloyd. *Labs. of The Rockefeller Inst. for Medical Research, New York City.* Single shocks were delivered to the brachial plexus of spinal cats to provide afferent activity reaching the spinal cord in the cervical enlargement. Activity in the lumbar enlargement resulting from such shocks was studied by means of microelectrodes and ventral root leads, using, as aids to analysis, segmental reflexes and cord lesions placed between the two enlargements. Since descending collaterals of the stimulated afferent fibers do not extend beyond the upper thoracic segments, long propriospinal neurons constitute the shortest pathway to the lumbar enlargement. The input to the lumbar enlargement has the attributes of a diffuse nuclear discharge rather than those of a synchronous volley.

The initial effect in the lumbar enlargement is inhibition of certain two-neuron arc reflex discharges. This inhibition, on the basis of latency (approximately 2.5 msec.), is a direct action mediated through the shortest pathways. Thus, direct inhibition results from internuncial activity as it does from primary afferent activity (Lloyd, J. Neurophysiol. **4**: 184). Inhibition is unilateral and ipsilateral.

Facilitation of lumbar motoneurons begins at approximately 7 msec. and lasts for 35 msec. Coincident with motoneuron facilitation internuncial activity may be recorded throughout the ventral horn. The dorsal horn is silent. Furthermore, dorsal horn activity, initiated by other means, is not conditioned. Excitatory pathways are almost equally crossed and uncrossed in contrast to the inhibitory pathway.

Motoneurons, ipsilateral and contralateral, discharge approximately 9–11 msec. after the brachial plexus shock. The reflex discharges are variable, but may approximate the size and synchrony of local reflexes.

Increased red blood cell fragility during lipemia. Joan Longini (by invitation), L. W. Freeman (by invitation) and Victor Johnson. *Dept. of Physiology, Univ. of Chicago.* In an attempt to determine whether sufficient hemolytic material (demonstrated to occur in thoracic duct lymph during absorption of a fat meal) enters the bloodstream to injure red blood cells, fragility studies were carried out on red cells exposed to fasting and to lipemic serum.

Equal volumes of whole blood and lipemic serum (from dogs) were shaken gently for 45 minutes. Then two volumes of water were added and shaking resumed for 30 minutes. Red cell counts were then made on the mixtures. The same procedure was carried out simultaneously on equal volumes of whole blood and fasting serum with later addition of distilled water. Practically invariably the counts on red blood cells exposed to lipemic serum (and subsequently distilled water) were significantly lower than counts on red blood cells exposed to fasting serum (plus distilled water). This suggests that the hemolytic agent in thoracic duct lymph during fat absorption enters the blood stream in sufficient quantities to increase the fragility of red blood cells, probably contributing to the normal daily destruction of red blood cells.

Cancellation of the electrical cochlear response by simultaneous stimulation with air- and bone-conducted sound. Karl Lowy (introduced by E. A. Culler). *Laby. of Psychology, Univ. of Rochester, N. Y.* In order to prove that air-borne and bone-conducted sound involve the same physical process within the cochlea, Békésy exposed a human subject to a pure tone by both air and bone conduction, simultaneously. By adjusting intensity and phase, he succeeded, under certain conditions, in making the tone disappear. Wever and Bray came to the same conclusion by considering the similarity of the electrical response elicited by air and by bone conduction, respectively. Although the latter procedure does not seem to offer an absolute proof, it is based on entirely objective methods. Békésy's results, on the other hand, appear quite conclusive but are obtained by a

method involving the subjective impression of the person tested.

It can be shown that, under proper experimental conditions, the electrical response of the cat's ear can be greatly reduced by concurrent stimulation with bone and air conduction. Once the cancellation of the cochlear potential is obtained, the result is not influenced by changing the position of the active electrode, as could be observed in supplementary experiments on the cochlea of the guinea pig.

Sphincter mechanisms, smooth muscle motor-units and vascular patterns in the blood vessels of the retrolingual membrane of Rana pipiens. BRENTON R. LUTZ, GEORGE P. FULTON (by invitation) and JACK I. SELTZER (by invitation). *Dept. of Biology, Boston Univ., Boston, Mass.* (Demonstration). In the retrolingual membrane of Rana pipiens, with brain and medulla destroyed, the vascular pattern and the associated innervation were delineated, and mapped photographically, and the nature and distribution of the contractile perivascular elements were explored with a microelectrode. From two to four arterioles enter from each side, and ultimately divide into anastomosing capillaries which converge laterally into venules. Non-myelinated fibers form a loose gross plexus, which gives rise to a perivascular plexus consisting of wide-meshed loops continuous from arterioles to venules, and a fine network, sufficiently rich to innervate all the contractile elements, applied to the walls of the vessels, copious on the arterioles, sparse on the capillaries and venules. Evidence from stimulation of non-myelinated nerve fibers, recorded cinephotomicrographically, revealed 1, the presence of vascular smooth muscle motor-units, and 2, a sphincter mechanism, consisting of modified smooth muscle at the capillary origins, which functions independently to control blood flow locally. Brief faradic stimulation generally produced dilation followed by constriction in a limited vascular pattern, which was only a small portion of the total vascular area of the membrane. The area constricted was sometimes only a portion of that originally dilated. In addition, activity was confined frequently to the few modified smooth muscle cells at the region of the capillary origin. Such a sphincter-like mechanism responded independently to nerve stimulation, and exhibited spontaneous independent rhythmicity.

A critique of various methods for the bioassay of dihydrotachysterol (A. T. 10). EVAN W. MCCHESNEY (introduced by O. W. Barlow). *Research Labs., Winthrop Chemical Co., Inc., Rensselaer, N. Y.* The accepted method for the assay of A.T.10 is based on the "toxic borderline dose" technique of Holtz et al. (Biochem. Ztschr. **237**: 247, 1931). We have felt, however, that this method leaves something to be desired both as to its specificity and as to the consistency of the results. More specific techniques would be those based on a direct measurement of the hypercalcemia which A.T.10 produces in experimental animals.

Using the albino rat as a subject and within the limits of 0 to 7.5 mgm. A.T.10 per animal as a single dose, there is an approximately linear relationship between log dose and the peak increase in serum calcium. Based on this line, the error of a series of 10 observations, using 15 to 25 animals to a group, ranges from 0 43 per cent with an average of 15 per cent. Results obtained with smaller groups of animals are less reliable.

Assays of A.T.10 based on the hypercalcemia it produces in dogs are not entirely satisfactory. Preliminary work in this case indicated a linear relationship between dose and the increase in serum calcium. However, as the number of observations was increased, such great irregularities of individual response were found as to make it doubtful whether this procedure will be of any value for assay purposes unless prohibitively large numbers of animals are used.

Effect of anticoagulants on the specific gravity of blood and plasma. PAUL L. MCLAIN, GEORGE J. PASTORIUS and C. H. WILLIAM RUHE (introduced by C. C. Guthrie). *Dept. of Physiology and Pharmacology, Univ. of Pittsburgh School of Medicine, Pittsburgh, Pa.* Specific gravity determinations at 25°C./25°C. were made by the falling drop method on small samples of untreated, defibrinated, citrated, heparinized, and oxalated arterial blood from each of 11 rabbits. The corresponding plasma (or serum) specific gravities were also determined. The concentrations of the anticoagulants per cc. of whole blood were: sodium citrate, 5 mgm.; solution of heparin (Connaught), 0.01 cc.; potassium oxalate, 2 mgm.

Defibrination did not significantly alter the specific gravity of whole blood. Heparin lowered the specific gravity of blood significantly in a majority of instances (average change, 0.0007), but raised that of plasma slightly (average change, 0.0003). Oxalate raised the specific gravities of both blood and plasma, the average changes being 0.0008 and 0.0012, respectively. Citrate caused the most profound changes, elevating the specific gravity of blood, on the average, 0.0022, and that of plasma, 0.0037. The specific gravities of the red corpuscles (calculated) were consistently elevated by oxalate and citrate, and lowered by heparin. Hematocrit determinations corroborated the trend of blood specific gravity measurements.

The results indicate that, of the methods studied, defibrination or the addition of heparin solution are the most suitable to prevent alterations of the normal specific gravity of blood, and especially, of plasma.

Influence of heparin on toxicity of ouabain and digitalis. DAVID I. MACHT. *Pharmacological Research Lab., Hynson, Westcott & Dunning, Inc., Baltimore, Md.* (Read by title.) The potency of heparin prepared in these laboratories is invariably assayed on freshly drawn cat's arterial blood. Solutions of ouabain for therapeutic use prepared here are also assayed by the cat method. These circumstances prompted an inquiry as to whether combined administration of the two drugs would effect synergistic phenomena. Accordingly a solution of ouabain was assayed on one series of cats and then reassayed on another series, which received full doses of heparin just before ouabain injections were begun. It was discovered that the toxicity of ouabain was greatly diminished in heparinized cats, the m.l.d. being 50 per cent greater than that for the controls. Similar experiments with digitalis tincture also yielded higher readings in heparinized cats. Analyzed statistically, this difference in toxicity proved to be significant. The same results were obtained with injections of ouabain and digitalis solutions in which heparin had been dissolved. To elucidate this phenomenon blood samples from cats were taken *before* and *repeatedly after* injection of heart drugs. These experiments revealed a progressive shortening in clotting time of blood of normal cats, but the typical prolongation or delay of clotting in samples from heparinized animals. The present findings suggest the possibility of minute intravascular clots after administration of large doses of digitaloids and might serve to explain the cases of sudden death occasionally reported after intravenous injections of ouabain. The clinical and therapeutic implications of these findings are obvious.

Comparison of cobra venom and opiates on basal metabolism of man. DAVID I. MACHT. *Pharmacological Research Laby., Hynson, Westcott & Dunning, Inc., Baltimore, Md.* (Read by title.) Studies on basal metabolism were made on five normal human subjects. The apparatus employed was the latest model of the Jones Motor-Basal Metabolism Unit. Cobra venom solution, practically free from proteins and hemotoxic ingredients and consisting virtually of cobra neurotoxin, was administered by intramuscular injection in doses of from 5 to 10 mouse units. Readings were made one to two hours later. The cobra venom injections were never depressant. On the contrary, the subjects were usually stimulated psychically and their vision, hearing and olfaction became more acute. The metabolism curve indicated either no effect whatever or a slight increase in metabolism rate. Control experiments in the same subjects with either morphine ($\frac{1}{10}$ to $\frac{1}{8}$ gr.) or codeine ($\frac{1}{2}$ gr.) definitely retarded the rate of respiration and effected an accumulation of CO_2 in the blood causing deeper respiratory movements. This partial asphyxia with resultant deep and slow breathing excursions distorted the tracings obtained with the apparatus employed and rendered their interpretation more difficult with regard to the effect on metabolism.

Absorption of epinephrine through the bone marrow. DAVID I. MACHT. *Pharmacological Research Laby., Hynson, Westcott & Dunning, Inc., Baltimore, Md.* (Read by title.) The effect of administration of epinephrine, in both aqueous and oil solutions, through various portals of entry, was studied on guinea pigs, rabbits and cats. Intramuscular injection of the aqueous solutions produced little or no rise in blood pressure of cats and rabbits. Intravenous injections effected the usual immediate and high rise in blood pressure, which rapidly disappeared and was followed by a compensatory fall of the pressure to below the normal level. Aqueous solutions of epinephrine, injected into the medullary cavity of long bones were rapidly absorbed and effected a blood pressure picture which could hardly be distinguished from that obtained after intravenous injection. A different effect was obtained with solutions or suspensions of epinephrine in fixed oils. Intramuscular injections produced little change in the blood pressure level of rabbits and cats or, at most, a rise of but a few millimeters. Injection of epinephrine oil suspensions into the medullary cavities of the tibia or humerus, however, was followed by a marked and sustained rise in blood pressure, lasting from 10 to 30 minutes. Control experiments with various pharmacologically inert fixed oils such as olive oil, peanut, peach kernel and mineral oils, slowly injected into medullary cavities, were usually harmless. Dangerous oil embolism occurred rarely. Fixed oils containing epinephrine, when introduced into the bone cavities, apparently act as a reservoir from which the active principle is slowly absorbed, producing a prolonged pharmacological effect.

Effect of intramedullary injections of purgative and other oils. DAVID I. MACHT. *Pharmacological Research Laby., Hynson, Westcott & Dunning, Inc., Baltimore, Md.* (Read by title.) In connection with an extensive study concerning absorption of drugs through the bone marrow, the writer studied the effects of various fixed and volatile oils. The fixed oils examined were olive oil, mineral oil, peach kernel oil, peanut oil, lard oil, cod liver oil, linseed oil and turtle oil. Small quantities, 0.1 to 0.5 cc. of such oils, introduced into the tibia or femur of guinea pigs, rabbits and cats, produced no deleterious effect. Deaths ascribable to oil embolism were rarely noted. When active drugs were dissolved in such oils, however, their characteristic pharmacological properties slowly developed. Thus camphorated oil produced convul-

sions. Of special interest were experiments with some laxative oils. Mineral oil injections had no laxative action. Castor oil in small quantities (0.2 to 0.5 cc.) also had no laxative effect, thus confirming the usual conception with regard to its purgative mechanism, which postulates previous formation of ricinoleic acid as the active agent. Croton oil, even in minute quantities, 0.1 or 0.2 cc. of 1:10 dilutions in olive oil, promptly produced purgation followed usually by ejaculations of urine and semen. Coma, convulsions and death followed excessive doses. Intramedullar injections of *Ruvettus* oil (J. Am. Pharm. Assoc. **XX:** 558, 1931) were mildly laxative. All the volatile oils, in contrast to the fixed oils, were rapidly absorbed through the bone marrow and produced depression of the central nervous system, coma, convulsions and death, depending on the dosage employed.

Effect of physical agents on stability of heparin. DAVID I. MACHT. *Pharmacological Research Laby., Hynson, Westcott & Dunning, Inc., Baltimore, Md.* (Read by title.) Repeatedly purified and highly potent heparin, prepared in these laboratories from livers of dogs and lungs of cattle, was dissolved in physiological sodium chloride solution and the effects of various physical agents on its antithromboplastic or anticoagulant property were studied over various periods of time. The effect of the following physical factors was investigated: 1, heating in an autoclave for 20 minutes under 15 pounds of pressure; 2, prolonged exposure of both the solutions and powdered heparin to sunlight for many days; 3, exposure to polarized light of the visible spectrum; 4, irradiation with ultraviolet rays from a quartz mercury vapor lamp; 5, exposure to x-rays; 6, exposure to radium emanations; 7, agitation by mechanical vibrations; 8, subjection to extremely high mechanical pressures. The samples were repeatedly tested before and after treatment by Howell's cat blood method. None of the physical agents, with the exception of x-rays and radium, in any way impaired the anticoagulating properties of the heparin. Exposure to 1000 r of x-rays and 1000 gamma ray units of radium energy, respectively, slightly reduced its anticoagulant effect.

Duration of heparin effect in relation to method of administration. DAVID I. MACHT. *Pharmacological Research Laby., Hynson, Westcott & Dunning, Inc., Baltimore, Md.* (Read by title.) Highly potent and repeatedly purified heparin, prepared in these laboratories from livers of dogs and lungs of beeves, was administered to rabbits and cats and occasionally to dogs in various ways, and the duration of its anticoagulant effect was studied on whole arterial blood by Howell's method. Subcutaneous or intramuscular injections of saline solutions were ineffectual, the coagulation of blood being prolonged only when it was drawn within 15 minutes after administration. Intravenous injections markedly delayed coagulation time. This effect could be detected, however, usually only in samples drawn not later than one hour after injection. The heparin effect was fleeting even when twice as much of the drug was injected as was required for experiments in vitro. Sublingual applications of pure heparin did not noticeably affect the clotting time. Heparin effectiveness was prolonged, however, by injecting it, suspended in olive or peanut oil, into the medullary cavities of the tibia, femur or humerus. These oil suspensions served as a sort of reservoir for the drug from which it was slowly eliminated and absorbed, thus exerting its anticoagulant effect over a period two or three times longer than it had in case of intravenous injections of aqueous solutions. Control experiments with olive and peanut oils proved harmless and serious complications due to oil embolism were very infrequently encountered after the intramedullary injections mentioned above.

Influence of tuberculin on behavior of rats and fishes. DAVID I. MACHT and LUCEILLE LISTON (by invitation). *Pharmacological Research Laby., Hynson, Westcott & Dunning, Inc., Baltimore, Md.* (Read by title.) Do the toxins of tubercle bacilli exert a stimulating influence on the brain and central nervous system and modify the personality, thus explaining the "spes phthisica" and characteristically optimistic attitude, euphoria and frequently extraordinary keenness of intellect exhibited by many consumptives, including famous historical personages?

To shed some light on the subject, experiments were undertaken on the behavior of goldfish, *Carassius auratus*, and of white rats. Rats were trained to run Watson's circular maze in the shortest possible time with but few or no errors. Intraperitoneal injections of old tuberculin (1:100) in doses of from 0.1 to 0.5 cc. produced either no effect or, more usually, definitely *stimulated* the behavior of the rats as indicated by a shortening of the running time and diminution of the number of errors. Although quite large, the doses injected produced no immediate or delayed depression. Ichthyometric studies (for method see J. Am. Pharm. Assoc. **XXX:** 203, 1941) on goldfish with solutions ranging from 1:100,000 to 1:300 also revealed no depression of their behavior. On the contrary, concentrations of 1:20,000 to 1:60,000 usually definitely stimulated the fish as indicated by greater frequency and wider amplitude of the waves recorded with the ichthyometer.

Pharmacodynamic synergism of cobra venom and thiamin. DAVID I. MACHT and ELIZABETH C. SPENCER (by invitation). *Pharmacological Research Laby., Hynson, Westcott & Dunning, Inc.,*

Baltimore, Md. (Read by title.) Cobra venom is primarily a neurotoxin acting on the central nervous system. Thiamin is known as the "nerve vitamin." A study of the two in combination was undertaken. A solution of cobra venom (H. W. & D.) freed from hemotoxic elements and proteins was assayed on white mice maintained on a standard balanced diet, and the m.l.d.$_{66}$ was established. Injection of such mice with 4 to 8 international units of thiamin for three or four days previously reduced the toxicity of cobra venom as much as 50 per cent. When larger doses (10 to 20 international units) of thiamin had thus been daily injected, however, the mice became more susceptible to cobra venom and the mortality rate rose greatly, the animals succumbing to doses smaller than the m.l.d. of controls. Mice fed on a diet deficient in thiamin were found to be very susceptible to cobra venom, the mortality rate being increased 50 per cent. Studies on the pain threshold of rats by the Macht method (J. Am. Pharm. Assoc., Scientific Section, **29:** 193, 1940) revealed that when the animals were kept on a diet deficient in vitamin B_1, they became more sensitive to pain stimuli than the controls. This vitamin deficiency did not antagonize the analgesic effect of cobra neurotoxin. On the contrary, smaller doses of the drug were effectual in raising the pain threshold.

Effect of sulfanilamide derivatives on phytotoxic reactions of blood. DAVID I. MACHT and ELIZABETH C. SPENCER (by invitation). *Pharmacological Research Laby., Hynson, Westcott & Dunning, Inc., Baltimore, Md.* (Read by title.) All the sulfanilamide derivatives are known to effect reactions, among which dermatological manifestations are particularly common. Urticaria, erythemata, morbilliform and other eruptions have been described. More recently, a pemphigoid dermatitis was reported after administration of sulfathiazole. Inasmuch as the Macht-Pels test is a reliable diagnostic procedure for differentiating true pemphigus from dermatoses simulating it, the writers investigated the phytotoxic reactions of blood sera from animals and human patients after administration of various sulfa compounds. Animal experiments were made on rabbits by administering doses of 0.5 to 1.0 gram of the respective sulfa derivatives by stomach tube and drawing blood samples six and twenty-four hours afterward. The compounds studied were sulfanilamide, sulfapyridine, sulfathiazole, sulfadiazine, sulfaguanidine, sulfasuccinamide and neoprontosil. Examination of the blood sera revealed that the drugs exerted no phytotoxic effect. On the contrary, all the compounds gave consistently higher phytotoxic indices (often ranging from 80 to 90 per cent) than normal rabbit's blood serum (ranging from 70 to 75 per cent). Blood specimens from patients treated with various sulfa compounds yielded results identical with those obtained from rabbits. Experiments in vitro with solutions of the drugs, 1:10,000, in plant-physiological saline on growth of *Lupinus albus* roots revealed very little toxicity. These findings are of interest in connection with the already highly complicated problem regarding the mechanism of the remarkable chemotherapeutic action of sulfanilamide and its derivatives.

Thromboplastic properties of some digitaloid drugs. DAVID I. MACHT, ELIZABETH C. SPENCER (by invitation) and LUCEILLE LISTON (by invitation). *Pharmacological Research Laby., Hynson, Westcott & Dunning, Inc., Baltimore, Md.* (Read by title.) The effect of small doses of various digitaloid principles in saline solution was studied by Howell's method on the clotting of whole blood from dogs, rabbits and cats. Various concentrations of the drugs, ranging from 1:500 to 1:20,000, were employed. The compounds examined were digitalein, digitalin, digitoxin, digitonin, digilanid, strophanthin, ouabain, convallamarin, scillaren and bufagin. All these glucosides were found to shorten the coagulation time markedly. Only digitonin and one sample of digitalin, probably contaminated, induced hemolysis. Control experiments with the glucosides esculin and salicin, as well as with a large number of different alkaloids in suitable concentrations, produced no such effect with the exception of epinephrine, which definitely shortened coagulation time. Experiments in vivo were made on cats with tincture of digitalis (1:10) and with ouabain (1:100-000). Samples of arterial blood, obtained in the course of the experiment, indicated progressive shortening of the coagulation time due to injections of the heart drugs even during the so-called therapeutic stages. Experiments with digitalis, administered by stomach tube to rabbits, are now in progress and their results appear to corroborate the findings obtained by intravenous administration of the drug.

Studies on decerebrate cats in the chronic state. MARTIN B. MACHT (by invitation) and PHILIP BARD. *Dept. of Physiology, School of Medicine, Johns Hopkins Univ., Baltimore, Md.* (Motion picture demonstration.) The animals to be exhibited were decerebrated under rigorous aseptic conditions at various levels between the caudel extremity of the diencephalon and the rostral border of the pons. In all cases an island of tissue consisting of hypothalamus and pituitary body and neurally isolated from the remaining brain stem was left in place with its blood supply impaired as little as possible. The post-operative survival times of the eight animals prepared in this manner have averaged to date more than 35 days and one of the cats shown lived 64 days.

Although these studies are still in progress, the following facts, most of which are illustrated in the film, seem to be definitely established: 1. Typical quadrupedal walking and effective righting depend upon mesencephalic mechanisms. 2. Only fragmentary expressions of rage can be evoked in bulbospinal and mesencephalic cats; compared to exhibitions of anger shown by hypothalamic and decorticate preparations, these responses are definitely incomplete. 3. An isolated island of tissue composed of hypothalamus and pituitary body is capable of preventing polyuria provided its blood supply is adequate (see Sumwalt, Erb and Bazett, Am. J. Physiol. **112:** 386, 1935). 4. Imperfect shivering and panting can be obtained in the mesencephalic cat at extremely low and extremely high rectal temperatures, respectively. 5. Conditioned eyelid responses to an auditory stimulus can be obtained in the mesencephalic cat, but they are not retained.

A number of responses, hitherto not reported for decerebrate preparations, are shown in the film.

The relation of the level of decerebration to the type of response evoked by faradic stimulation of the anterior lobe of the cerebellum in the decerebrate cat. MARTIN B. MACHT (introduced by Philip Bard). *Dept. of Physiology, School of Medicine, Johns Hopkins Univ., Baltimore, Md.* (Read by title.) The responses produced by electrical stimulation of the anterior lobe of the cerebellum may be divided into two main groups. We have observed a relationship between the type of response produced by stimulation (Harvard inductorium) and the level of decerebration. Fifty-two cats were used; in 22 the brainstem was severed through the inferior colliculi and the rostral border of the pons, and in 30 the transection passed through the superior colliculi and the caudal border of the mammillary bodies.

In the 22 animals with the lower transections, stimulation by electrodes on the surface of the anterior lobe produced variations of a response which typically involved all four legs. This consisted of *a*, flexion of both ipsilateral limbs and extension of the contralateral ones during stimulation, and *b*, extension of ipsilateral and flexion of contralateral limbs occurring as a rebound. This diphasic response could be evoked in the 30 cats with the higher transections, but with less certainty.

In 26 of the 30 animals with the higher transections a response involving principally the ipsilateral foreleg and consisting of either relaxation of rigidity or increased extension during stimulation and tonic flexion of this limb as a rebound could be elicited. This response was never seen in the animals with the lower transections.

It is concluded that the response involving all four limbs has no relationship to the level of decerebration while that involving principally the ipsilateral foreleg is dependent upon the integrity of mesencephalic mechanisms.

Factors affecting the vasomotor tone in the hindlimb muscles of the dog. J. P. MAES (Fellow of the Belgian American Educational Foundation) and J. R. PAPPENHEIMER (introduced by Magnus I. Gregersen). *Dept. of Physiology, College of Physicians and Surgeons, Columbia Univ., New York City.* In dogs anesthetized with chloralose or with nembutal the hindlimb muscles were provided with an independent circulation of defibrinated blood from a pump-lung preparation. The perfusion pressure of the blood supplying the muscles could be adjusted to any constant value. Connections of the muscles with the central nervous system were retained through the sciatic nerve. Circulatory changes in the animal therefore affected the flow through the muscles only by changes of vasomotor activity. At intervals the sciatic nerve was reversibly blocked by cold and the resulting changes of blood flow measured at different perfusion pressures. The ratio of the slopes of the pressure-flow curves, obtained with the nerve blocked and with the nerve active, was used as a quantitative measure of the vasomotor tone. The following factors affecting the vasomotor tone in the muscles have been investigated: hemorrhage, clamping the carotid arteries, administration of amyl nitrite, acetylcholine, adrenalin and reduction of the oxygen saturation of the blood.

Further investigation of the training pattern of human muscle. GEORGE MAISON and GEORGE KOTALIK (by invitation). *Dept. of Physiology, Wayne Univ. College of Medicine, Detroit.* A previous study (Am. J. Physiol. **132:** 390, 1941) showed that prolonged exercise with loads light enough to be carried in steady state accelerated the training process in human digital extensor muscle. It is now possible to define more clearly this effect.

The method employed a fixed extent and frequency of contraction with variable load. Subjects worked to fatigue once daily with a test load, and after an interval spent an additional 10 min. in steady state at the highest work rate they could easily maintain. Others have been trained without the additional exercise period.

Endurance (ability to maintain high work rate through appreciable time) was gained more continuously and rapidly in the exercised group. In the non-exercised group steady state at 8.4 kgm. cm. per sec. was gained in 90 days by only 3 muscles out of 15. In the exercised group this ability was gained in the same period by 11 of 18. Maximum steady-state-work-rate after prolonged

training in the former group was about ⅓ smaller than in the latter.

That function of absolute muscle power which can be measured in the intact subject showed small increases after long training. F. E., after 9 months during which the steady state maximum had been multiplied between 2 and 3 fold, the absolute power had increased *ca* 30 per cent.

With discontinuance of work, after one month, endurance depreciated rapidly to less than 50 per cent. However, absolute power remained at its high level for an undetermined time.

Further studies on shock with particular reference to electrolytes. J. F. MANERY, D. Y. SOLANDT and E. RANDELL (by invitation). *Depts. of Biochemistry and Physiological Hygiene, University of Toronto, Canada.* The condition of traumatic shock studied in these experiments is accompanied by a slight but not extensive hemorrhage at the site of injury. It is characterized by increased plasma potassium and decreased plasma chloride concentrations, slight hemodilution and a constant plasma water concentration.

Analyses of tissues showed that the injured muscle lost at least half of its normal potassium content. Some of this was recovered from the exudate found at the site of injury, while the remainder probably leaked out into the general circulation. To simulate this condition, normal intact dogs were subjected to a continuous injection of a solution of potassium administered at such a rate that they succumbed in five to ten hours. The amount of potassium required to kill the animal was at least ten times that liberated from the injured muscle. Much of this was excreted but even the amount retained exceeded that lost by the muscle. The characteristics of traumatic and hemorrhage shock were compared to the condition produced by a slow continuous injection of potassium.

The distribution and nature of ganglionic inhibition in the sympathetic system. AMEDEO S. MARRAZZI (introduced by George B. Wallace). *Dept. of Pharmacology, New York Univ. College of Medicine, New York City.* In previous communications we have dealt with the superior cervical ganglion and tentatively generalized the adrenergic inhibition there observed to apply to other sympathetic ganglia.

By stimulating the inferior splanchnic nerves and recording the reduction in action potentials in the hypogastric nerve when adrenaline is injected intravenously, the generalization is shown to hold for the inferior mesenteric ganglion. Likewise, by stimulating the anterior roots (thoracic 13—lumbar 3) and recording the reduction in sympathetic potentials in the sciatic nerve on injecting adrenaline intravenously, adrenergic inhibition in the ganglia of the lateral sympathetic chain is established. Thus the phenomenon is demonstrated in all types of sympathetic ganglia, interrupting visceral and somatic efferents.

In the experiments originally described the dissection was such as to limit the blood flow, bearing injected or liberated adrenaline, to the ganglion, excluding the pre and postganglionic nerves from their supply. On this basis and because adrenaline did not alter the natural discharge of impulses in preganglionic nerves, the action was localized to the ganglionic synapses. Since that time a differential input to the amplifiers has made it possible regularly to distinguish in the hypogastric nerve potentials, on stimulating the preganglionic splanchnics, the D waves of Lloyd assignable to "straight-through" preganglionic fibers continuing into the hypogastric without synapsing in the inferior mesenteric ganglion, as well as the usual postganglionic waves. Adrenaline, while reducing the postsynaptic potentials, leaves the D, or preganglionic, potentials intact affording additional evidence that the preganglionic fibers are not affected and that the action is synaptic. [*Aided by a grant from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association.*]

Phosphorus metabolism of nuclei. ALFRED MARSHAK. *Radiation Laby., Univ. of California, Berkeley.* Nuclei of liver and tumor tissue are obtained free of cytoplasm by centrifugation after treatment with dilute citric acid. Radioactive phosphorus is taken up by nuclei of cells which do not undergo mitosis as well as by those which do. Tumor nuclei accumulate P_{32}, normal liver nuclei do not. In normal liver the relative concentrations of P_{32} in nucleus and cytoplasm differ markedly from that of a carcinoma, but in regenerating liver the relative concentrations are the same as in the carcinoma. In normal liver nuclei 60–70 per cent of the nuclear P_{32} is in the nucleoprotein fraction, in a lymphoma 90–95 per cent is in this fraction. X-ray treatment alters the distribution of P_{32} between nucleus and cytoplasm. In nuclei which do not undergo mitosis there is continual breakdown and resynthesis of nucleic acid.

Facilitation and recruitment during the subnormal phase in the geniculo-striate system of the cat. WADE H. MARSHALL. *Laby. of Physiological Optics, Wilmer Ophthalmological Inst., School of Medicine, Johns Hopkins Univ.* During an interval of 10 to 30 msec. following a single conditioning shock of the optic nerve of the nembutalized cat, a single test shock gives an enhanced response. This is followed for a longer interval by a decreased response. Recruitment is maximum with weak shocks; with strong shocks little or no summation occurs. The former condition favors peaking of activity through the ascending primary

projection system, by the action of lateral and vertical components of summation in a system in which reciprocal overlap is present. With stronger shocks the neural system is saturated, and the vertical component becomes a plateau of activity instead of a peak.

If the subnormal phase produced by a brief volley is explored with paired shocks, a relatively greater degree of facilitation and recruitment is seen. Each response is smaller, and facilitation is present for stronger stimuli, than in the case discussed above. This means that the system is not saturated by strong stimuli in the subnormal phase, and since marked recruitment occurs, the peaking effect is preserved in the reciprocally overlapped system. The relative values of recruitment are greater for all surface-recorded cortical components than for any geniculate case thus far seen. This indicates a relatively greater degree of cascade facilitation through the ascending projection system, which also favors the peaking action of a train of stimuli in the subnormal phase.

Influence of temperature on respiration and glycolysis in isolated rabbit brain. ARTHUR W. MARTIN (by invitation), FREDERICK A. FUHRMAN (by invitation) and JOHN FIELD, 2ND. *Dept. of Physiology, Stanford Univ. and the Univ. of Washington.* The work reported here is part of a systematic survey of the influence of temperature, over a wide range, on respiration and glycolysis in various excised tissues and organs.

Eighteen rabbits were used. Oxygen consumption and anaerobic glycolysis were measured concomitantly on cerebral cortex slices from the same animal, using conventional manometric methods (Dixon). Two thermostats were available, so that in every case a run was made at 37.5°C. (for general reference) and at one other temperature level. The temperature range covered was 5° to 47.5°C. All runs were made in triplicate.

At all temperatures above 5°C., the rate of anaerobic glycolysis ($Q_{CO_2}^{N_2}$) was greater than the rate of respiration (Q_{O_2}). At 5° and at 45°C. the values of these measurements were nearly the same. Over the range 5°–45°, the differences between $Q_{CO_2}^{N_2}$ and Q_{O_2} were significant (P greater than 0.001 and less than 0.01, Fisher's T test). Over the range 5° to 25° the value of the difference ($Q_{CO_2}^{N_2}$–Q_{O_2}), *increased* with rise in temperature, while from 25° to 40° it was quite constant. The thermal optimum for $Q_{CO_2}^{N_2}$ was approximately 42.5°C; for Q_{O_2}, 45°C. [*Supported by grants from the Markle Foundation and from the Stanford University School of Medicine to one of us (J. F.)*]

The achromotrichial action of p-aminobenzoic acid. GUSTAV J. MARTIN (introduced by Walter Goldfarb). *Warner Inst. for Therapeutic Research, New York City.* In these experiments, p-aminobenzoic acid was found to have a vitamin-like action in rats on diets containing inositol and six other B complex factors (thiamine, riboflavin, pyridoxine, nicotinic acid, pantothenic acid, choline and inositol). The syndrome of p-aminobenzoic acid deficiency in these rats was characterized by a slight greying. However, in checking the chromotrichial factor, p-aminobenzoic acid was fed at different levels in diets supplemented with calcium pantothenate in varying amounts, with the following results:

DIET	CALCIUM PANTOTHENATE DAILY PER RAT	p-AMINOBENZOIC ACID DAILY PER RAT	GREYING
1	1.0 mg.	1.0 mg.	½+
2	1.0 mg.	250 μg.	1+
3	250 μg.	1.0 mg.	4+
4	1.0 mg.	5.0 mg.	6+
5	100 μg.	10 μg.	½+

6+ indicates complete greying.

It is concluded that the ratio of calcium pantothenate to p-aminobenzoic acid is the important factor; a ratio of calcium pantothenate to p-aminobenzoic acid favoring the latter, results in greying. It is not the absolute but the relative amounts of these factors which determines the incidence of achromotrichia.

It is suggested that the stimulation and/or inhibition of the growth of microorganisms in the gastro-intestinal tract determines the results seen. The probable explanation is that there is a destruction of pantothenic acid by microorganisms which are stimulated in their growth by p-aminobenzoic acid.

Extra and intracellular cH and the stimulating action of acetylcholine. ARNOLD MASON (by invitation) and ROBERT GESELL. *Dept. of Physiology, Univ. of Michigan.* The velocity of the well-known destruction of acetylcholine in the blood is readily demonstrable. Acetylcholine delayed 5 seconds in the occluded external jugular loses much of its depressant action on blood pressure. Sixty seconds delay may abolish all effects. This destruction is markedly accelerated by alkalinity. Decreasing contractions of muscle immersed in samples of blood plus acetylcholine adjusted in pH from approximately 5.5 to 8.5 indicate that highly significant changes in the destruction of acetylcholine must occur within the normal pH range of blood. The preservative action of acidity on acetylcholine in the *tissues* is indicated with the use of Ringer's solution. Previous acidification of muscle with CO_2 increases the height of its contraction when *momentarily* immersed in Ringer's solution plus acetylcholine. *Continued* immersion of muscle in carbonated Ringer's solution plus acetylcholine

produced a long continued increasing strength of contraction. As the acidity of muscle and motor synapse increases, the acetylcholine diffusing across the cell membrane is conceivably better preserved and, therefore, reaches higher concentrations. In a non-carbonated Ringer's solution contraction tends to reach a lower sustained level indicating that acetylcholine is being destroyed as rapidly as it enters the cell. Addition of NaCN to non-carbonated Ringer's solution plus acetylcholine in the midst of a steady-state contraction increased the height of that contraction. This may be due to an increase of intracellular cH caused by the rapid formation of lactic acid.

Intercompatible reactions to blood transfusion related to the donor's diet. N. A. MASOR (by invitation), HARRY GREENGARD, W. L. VOEGTLIN (by invitation), J. P. SANDBLOM (by invitation) and A. C. IVY. *Dept. of Physiology and Pharmacology, Northwestern Univ. Medical School, Chicago.* Donor and recipient dogs were grouped into mutually compatible pairs on the basis of microscopic cross-agglutination tests. The unanesthetized donor (local anesthesia) was bled to the extent of 200–300 cc., and this blood, untreated, was injected into the anesthetized and unanesthetized recipients. Prior to the transfusion the donor animals were either fasted or fed a fat, protein, carbohydrate, or protein-carbohydrate meal; similar treatment was accorded the recipients. A record of the recipient's carotid blood pressure was taken during the transfusion and for at least 30 minutes thereafter; a transfusion reaction was evidenced by a marked drop in blood pressure, beginning 3–5 minutes after the transfusion was completed and lasting 20–45 minutes, with general evidences of shock and gradual recovery. Such reactions were most frequently obtained in fasted recipients when the donor was fed a protein or carbohydrate-protein meal, less frequently in the case of a carbohydrate-fed or fasted donor; and not in any case when the donor was fed fat. Likewise, no reaction has been noted when both donor and recipient dogs were fed. These observations are based on 160 transfusions.

Factors regulating the lipid content of the rat endometrium. G. MASSON (by invitation) and HANS SELYE. *Dept. of Anatomy, McGill Univ., Montreal, Canada.* It has been stressed that in lactating rats a dense layer of lipid granules accumulates in the basal part of the endometrial epithelium (Selye and McKeown, Proc. Roy. Soc. London **119**: 1, 1935). Recent investigations show that moderate quantities of lipid granules are always seen in this region in recently spayed rats, but disappear several weeks after ovariectomy at a time when endometrial atrophy becomes severe. "Folliculoid" compounds, such as estradiol, cause immediate disappearance of these lipid granules in recently spayed rats, while progesterone, desoxycorticosterone acetate, methyl testosterone and testosterone may cause marked uterine enlargement without depleting the endometrium of its lipid granules. Progesterone does not inhibit the lipid depleting effect of simultaneously administered estradiol although it counteracts its uterus enlarging effect. Δ^5-dehydro-*iso*-androsterone and its acetate which exhibit a definite folliculoid effect (as judged by the keratinization of the vagina) resemble estradiol inasmuch as they also free the uterine epithelium of its lipids. A systematic study of numerous naturally occurring and synthetic steroids indicates that only those endowed with marked folliculoid action have the power to deplete the endometrium of its lipid granules. Hence this effect appears to be specific for folliculoid compounds.

The effect of phosphorus on metabolism in *Chilomonas paramecium*. S. O. MAST and D. M. PACE (by invitation). *The Zoological Laby. of The Johns Hopkins Univ.* (Read by title.) *Chilomonas paramecium* grows indefinitely in isolation or mass cultures in water containing $KC_2H_3O_4$, Na_2HPO_4, NH_4Cl, $(NH_4)SO_4$, $MgCl_2$, and $CaCl_2$ but it dies within a few days if phosphorus is omitted.

If the concentration of Na_2HPO_4 is increased from zero the rate of reproduction in isolation cultures increases from nearly zero to a maximum average of 4 divisions per day at 0.00109 M Na_2HPO_4, and then decreases to zero at 0.0035 M. If Na_2SiO_3 is added the rate of reproduction increases from nearly zero at 0.0 M Na_2HPO_4 to a maximum of 4.133 division per day at 0.00101 M Na_2HPO_4 and then decreases to zero at 0.0035 M.

If phosphorus is omitted and the concentration of Na_2SiO_3 increases, the rate of reproduction increases to a maximum at 0.001 M Na_2SiO_3 and then decreases to zero. If phosphorus is added, the optimum concentration of Na_2SiO_3 is considerably lower.

If phosphorus is omitted and Na_2SiO_3 added in optimum concentration the chilomonads die out in about 10 days but they divide more frequently and live several days longer than they do if no Na_2SiO_3 is added.

The sodium in the Na_2SiO_3 and the Na_2HPO_4 has no measurable effect on *Chilomonas*.

Phosphorus is therefore necessary for growth in *Chilomonas* and silicon can not take its place but it reduces the optimum concentration of phosphorus and increases the rate of reproduction at the optimum and the length of life without phosphorus.

Absence of phosphorus has no direct effect on synthesis of starch but it causes marked accumulation of fat.

Studies on gravity shock in the dog. H. S. MAYER-

SON. *Dept. of Physiology, Tulane Univ. School of Medicine, New Orleans, La.* Dogs, anesthetized with chloralose or Na barbital and suspended in the upright position for relatively long periods of time, show a loss of compensatory ability and failure of the circulation particularly if the anesthesia is deep or the carotid sinus and cardio-aortic nerves are sectioned. Accompanying the hypotension (arterial pressure of 60 mm. Hg or less) there is hemoconcentration, slowing of blood flow and increased capillary permeability. The arterio-venous oxygen difference of femoral blood increases (50 to 100 p.c.) as does the venous (jugular and femoral) CO_2 content. Decrease in effective circulating blood volume is also indicated by the fact that the withdrawal of 5 to 15 cc. of blood frequently precipitates immediate death. Thoracic lymph protein increases at the expense of plasma protein. If the animal is returned to the horizontal position before irreversible failure has occurred, there is a marked and persistent increase (100 to 300 p.c.) in the flow of lymph containing large amounts of protein. Assays of blood and lymph (2 experiments) show no significant changes in histamine concentration.

These preliminary findings suggest that this procedure offers a convenient and readily controlled method of inducing a state of shock which permits the study of the sequence and interrelationships of the various events that occur. It would also seem to provide a simple procedure to test the efficacy of some forms of suggested shock therapy. [*Aided by a grant from the John and Mary R. Markle Foundation.*]

The influence of anesthesia and cortical lesions on vestibular nystagmus in the monkey. JEROME K. MERLIS (Commonwealth Fund Fellow from the University of Louisville) (introduced by J. F. Fulton). *Laby. of Physiology, Yale Univ. School of Medicine, New Haven, Conn.* (Read by title.) Conjugate deviation of the eyes has been reported following lesions of certain areas of the cerebral cortex. Monkeys subjected to such operations show, during the postoperative recovery period, a modified form of postrotatory and caloric nystagmus not encountered in the normal. For a week, or longer, following ablation of the frontal eye field (area 8 of Brodmann), or of angular gyrus, the fast component of postrotatory or caloric nystagmus was diminished or absent, the reaction consisting of maintained deviation of the eyes in the direction of the slow component, or bursts of essentially normal nystagmus with intervals of such deviation. There was no correlation with the side of the lesion or the spatial orientation of the head.

Similar changes were observed in two monkeys whose temporal lobes were ablated, and in two in whom no cortical lesions were made, but which had had intracranial section of the optic nerves at the chiasma.

The influence of the anesthetic itself was studied by responses of a normal monkey under the full anesthetic dose. Sodium amytal, 0.60 gm/kgm, was injected, half intraperitoneally, half intramuscularly. Within three minutes, the fast component diminished, disappearing completely in eight minutes. For three days following the animal had the modified nystagmus observed postoperatively in other monkeys of the series.

Since the changes in nystagmus may be accounted for on the basis of prolonged postanesthetic effect, none of the changes observed can be specifically ascribed to lesions of the eye-motor areas of the cerebral cortex.

The effect of ablation of the cerebellum on the cortically induced contractions of a pair of antagonistic muscles in the monkey. JEROME K. MERLIS (Commonwealth Fund Fellow from the University of Louisville) and THOMAS A. FRASER (Ellen Micle Fellow of the University of Toronto) (introduced by J. F. Fulton). *Laby. of Physiology, Yale Univ. School of Medicine, New Haven, Conn.* (Read by title.) In the macaque, the tension curves of a pair of antagonistic muscles, soleus and tibialis anticus, in response to electrical stimulation of the motor area of the cerebral cortex (area 4 of Brodmann), were studied with optically recording isometric torsion wire myographs. In the motor cortex, foci were located which, upon stimulation, provoked reciprocal responses of the two muscles, e.g., contraction of tibialis and concurrent relaxation of soleus. After establishing the characteristic response for a given focus, the cerebellum was completely ablated, and responses from the same focus again recorded.

Immediately after decerebellation in three monkeys the following changes were noted: 1, a decreased responsiveness to cortical stimulation, i.e., a stronger stimulus was required to provoke a response; 2, augmentation of the extensor responses, and depression of the flexor responses; 3, reciprocal contraction-relaxation responses were absent.

In one other monkey similar changes occurred, but only after the lapse of 12 hours postoperatively. Immediately after decerebellation in this animal the response of the two muscles could not be distinguished from the control tension curves. These findings suggest the possibility that the cerebellum does not exert a tonic influence responsible for the reciprocal nature of the response of a pair of antagonists. The delayed changes in the direction of extensor augmentation in this animal, and the similar changes of more immediate onset in the other three, may possibly be attributable to extraneous factors introduced by the

operation of cerebellar ablation, such as edema or vascular disturbance.

Reticulocytosis as a sign of damage of the frontal lobe of the brain. FRED A. METTLER. *Dept. of Neurology, College of Physicians and Surgeons, Columbia Univ.* (Read by title.) Evidence of various types of autonomic imbalance following removal of different portions of the cerebral cortex has been previously presented by the author. Most of these autonomic dysfunctions have been found to be caused by injury to the frontal cortex rather than other areas. Frontal lobe injury (dogs) also produces a marked reticulocyte response which appears after a latent period of about four days, rapidly reaches a maximum and returns to normal levels within about two weeks. The reticulocyte concentration however remains essentially unstable and periodic storms of reticulocytosis are subsequently seen. Such a phenomenon is not to be explained upon the basis of operative blood loss nor opening of the cranial case since removal of other cortical areas does not produce it. It is not due to hemoconcentration for it is not directly related with postoperative weight loss nor is there hemoconcentration as indicated by erythrocyte count. The effect is seen when the operation has been strictly limited to the frontal cortex.

The ultraviolet irradiation of auto-transfused blood: Studies in combined oxygen values. G. MILEY (introduced by R. Beutner). *Dept. of Pharmacology, Hahnemann Medical College of Philadelphia.* The effects of 97 applications of Knott's technique of ultraviolet irradiation of auto-transfused blood in 70 individuals on combined oxygen values of venous blood have been studied and divided into three groups.

Group I. Combined oxygen values, taken just before irradiation of auto-transfusions, compared with those taken within 10 minutes following irradiations, showed in a series of 83 irradiated auto-transfusions an average combined oxygen value of 7.0 vol. per cent before and of 11.1 vol. per cent after irradiation, an average increase of 4.1 vol. per cent, a 58.6 per cent gain. The greatest rise was from 1.0 to 8.2 vol. per cent, the greatest fall from 13.6 to 11.2 vol. per cent.

Group II. Values obtained in 14 irradiated auto-transfusions before irradiation and ½ hour later showed an average combined oxygen value of 9.7 vol. per cent before and of 8.8 vol. per cent after irradiation, an average decrease of 0.9 vol. per cent, an average fall in value of 9.2 per cent. The greatest rise was from 2.0 to 6.5 vol. per cent, the greatest fall from 15.0 to 9.2 vol. per cent.

Group III. Combined oxygen values in 27 irradiated auto-transfusions with an average before irradiation of 5.8 vol. per cent, 1 month or more later was 8.7 vol. per cent, an average increase of 2.9 vol. per cent or 50 per cent. The greatest gain was from 3.0 to 17.9 vol. per cent, the greatest drop from 6.6 to 4.6 vol. per cent.

Erythrocyte and hemoglobin determinations were negative. In 60 out of 97 instances, skin pinkness persisted from 10 to 30 days, 6 individuals showed marked cyanosis which disappeared after irradiation.

Our findings suggests that the constant increase in the amount of oxygen found in venous blood shortly after reinjection of the individual's ultraviolet irradiated blood is due to an increased ability of the blood to combine with oxygen.

Pancreatic diabetes in the owl. I. ARTHUR MIRSKY, NORTON NELSON (by invitation) and SAMUEL ELGART (by invitation). *May Inst. for Medical Research, Jewish Hospital, Cincinnati, O.* In continuation of our studies on avian carbohydrate and fat metabolism, we studied the influence of pancreatic ablation from the horned owl. Whereas the depancreatized domestic duck reveals no significant rise in the blood sugar consequent to pancreatectomy, the depancreatized owl shows a marked rise in blood sugar. The correlation between the food habit of this species and the susceptibility to pancreatic diabetes is discussed.

Further study of the influence of activated sterols on blood pressure. RUDOLPH MRAZEK (by invitation), CLARENCE G. NOVAK (by invitation) and C. I. REED. *Univ. of Illinois, Chicago Colleges.* This work is an extension and confirmation of the report by Briskin before the Physiological Society in 1941 from which it is concluded that 1, The use of desoxycorticosterone proves that recording blood pressure from the tail of a rat is a valid and reliable method since hypertension was produced; 2, none of three different preparations of vitamin D produced hypertension even with toxication and death of the animals; 3, on the basis of fatalities produced the increasing order of toxicity for three preparations is ertron, vitamin D_2, vitamin D_3.

Similar studies on the dog are under way.

Selective accumulation of potassium by myosin. LORIN J. MULLINS (introduced by W. O. Fenn). *Dept. of Physiology, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.* Experiments have been carried out wherein myosin gel, which had been extracted from fresh muscle as the isoelectric protein, was immersed in isotonic equi-molal solutions of Na^+ and K^+ (total concentration 0.2 M). Phosphate buffer was used to maintain pH at the desired values; and, at suitable intervals, samples of the myosin could be removed from the solution by centrifugation and an analysis for Na^+ and K^+ carried out. These analyses showed that K^+ had been concentrated in the myosin by about 3.7 times while Na had

been concentrated only 1.1 times, the external value.

The effective binding of K^+ was enhanced by high pH values up to pH 8 and the ion uptake by the protein was markedly influenced by temperatures as low as 37°C. at which temperature the myosin has been observed to denature, and concomitantly to lose accumulated potassium.

That these phenomena are directly referable to differences in ion mobility between Na^+ and K^+ does not seem likely in view of the fact that the equilibrium, as followed by sampling at various times, appears to be 99 per cent complete in 1 hour and most experiments were carried out for 24 hours.

It is suggested that this property of myosin as described above, may form a basis for the explanation of the anomalous distribution of K between muscle and plasma.

Electrical potential and activity of cholinesterase in nerve. D. NACHMANSOHN. *Laby. of Physiology, Yale Univ. School of Medicine, New Haven, Conn.* Recent investigations suggest that acetylcholine metabolism is intrinsically connected with the electrical changes occurring everywhere at the neuronal surface during activity. Two facts are essential for the new concept: 1, the parallelism between E.M.F. and concentration of cholinesterase in electric organs, and 2, the localization of the enzyme at the neuronal surface. The new interpretation of the significance of acetylcholine agrees with the conclusions based on the electrical signs of nerve activity, i.e., that the difference between conduction along fibers and across synapses is only a quantitative one.

Investigations have been initiated to determine whether the localization inside the nerve cell and the parallelism between activity and E.M.F. are specific for cholinesterase.

Experiments on the giant fiber of the squid, with Dr. H. B. Steinbach, show that about 90 per cent of succinic dehydrogenase are located in the axoplasm and only 10 per cent in the sheath. The distribution of this enzyme, generally considered as an important link in respiration, is in striking contrast to that of cholinesterase. On the other hand the distribution of diphosphothiamin in the giant axon differs both from cholinesterase and succinic dehydrogenase. Its concentration in the sheath is several times as high as in the axoplasm. This appears significant since the acetylation of choline requires probably vitamin B_1, although this is certainly not the only function of this vitamin in intermediate cell metabolism.

Different enzyme activities are presently being compared in collaboration with Drs. Cox, Coates and Machado with electrical variations in the electric organ of *Electrophorus electricus*. [*Aided by a grant from the Dazian Foundation.*]

The nature of the S wave. L. H. NAHUM, H. E. HOFF and W. KAUFMAN (Fellow of the Dazian Foundation) (by invitation). SIII, when present, may be reduced or abolished by the application of M/5 KCl to the posterior surface of the left ventricle. Application of KCL to the anterior surface of the right ventricle increases the amplitude of SIII and eliminates its upstroke. It is concluded that the downstroke of SIII is caused by negativity at the posterior surface of the left ventricle which has not yet been balanced by completion of activation of the anterior surface of the right ventricle. Completion of activation of the anterior surface of the right ventricle causes the upstroke of SIII and establishes isoelectricity. Confirmation of these inferences is afforded by thermal applications to the interfering surfaces. Heating the anterior right and cooling the posterior left surfaces of the ventricles diminishes SIII, while cooling the anterior right and warming the posterior left surfaces increases SIII.

Extrasystoles elicited by electrical stimulation of the anterior surface of the left ventricle show S waves in lead III which react to KCl and to heat and cold exactly as does the Normal SIII.

SI is almost never found in the dog as prepared for those experiments, nor was it possible to elicit extrasystoles which consistently showed SI. [*Supported by a grant from the Fluid Research Fund, Yale University School of Medicine.*]

The effect of insulin on gastric secretion. H. NECHELES, W. H. OLSON and WILLIAM SCRUGGS. We have reported two years ago that the effect of insulin on gastric motility of dogs is usually biphasic; that is, a depression of gastric motility correlated with low blood sugars, and increased gastric motility usually correlated with a medium degree of hypoglycemia. (Am. J. Dig. Dis. 8: 270, 1941.) We have extended this work to the effects of insulin on gastric secretion, using Pavlow pouch dogs. In individual experiments on unfed dogs, less stimulation of gastric secretion was associated with higher doses of insulin and lower levels of blood sugar.

Following a meat meal, plus injection of insulin, secretion was distinctly depressed when low blood sugars prevailed.

The typical effect of insulin in provoking gastric secretion was correlated with mild hypoglycemia, while severe hypoglycemia depressed gastric secretion. It is important to note that in the experiments with low blood sugars the animals did not show any signs of subjective disturbance.

The relation between increased weights of the heart and adrenals in experimental hyperthyroidism in the rat. DOROTHY NELSON (introduced by F. T. Jung). *Dept. of Physiology and Pharmacology, Northwestern Univ. Medical School, Chicago.* (Read by title.) The growing recognition of the

similarity between certain of the toxic manifestations accompanying hyperthyroidism and the effects of adrenal insufficiency suggested the possibility of an important relationship between the size of the heart and the status of the adrenals.

Normal albino rats of both sexes and various ages were used for this investigation. The animals received daily constant amounts of desiccated thyroid (Wilson) containing two to six gamma iodine. The treatment periods were one, five, ten, and fifteen days. On the day following the last treatment, the animals were killed with ether. The hearts and adrenals were removed, freed under a dissectoscope of all extraneous tissue and fluid, and weighed.

To eliminate the spuriously large ratios between organ weight and body weight caused by the loss of body weight in the treated animals, the organ weights were expressed as milligrams per gram of initial body weight. Each group of animals was compared with untreated animals of the same age and sex.

Neither the heart nor the adrenals showed a consistent weight gain in the animals treated one or five days. The adrenals of the groups receiving thyroid containing six gamma of iodine daily reached a maximum weight increment in ten days, the others showing further gains in 15 days. The hearts were in all cases somewhat heavier with the larger doses and longer treatment. Space does not permit a presentation of the histologic changes at this time.

Effects of estrogenic hormone in thyroidectomized rats. WARREN O. NELSON. *Wayne Univ., College of Medicine, Detroit.* Earlier studies (Nelson and Hickman, 1937) have shown that estrin will correct the basophilism which occurs in the hypophyses of thyroidectomized rats. The present study extends these earlier experiments and offers comparisons between the effects of estrin on castrated, thyroidectomized and castrated-thyroidectomized female rats. A total of 178 animals were studied in the various experiments.

In animals subjected to treatment immediately after operation the daily amounts of estrin (estradiol benzoate) required to prevent basophilism for 35 days were 100, 300, and 300 I.U., respectively, for castrated, thyroidectomized, and castrate-thyroidectomized rats. In animals permitted to go untreated for 35 days after operation and subsequently treated for 20 days the amounts of estrin required to restore the percentage of basophiles to normal were 150, 300 and 300 I.U., respectively, for castrated, thyroidectomized, and castrate-thyroidectomized rats.

Although the basophilia of either castration or of thyroidectomy is prevented or corrected by estrin, there has been no evidence that such treatment effects any return of a normal acidophile population to the hypophyses of thyroidectomized animals.

The mammary glands and ovaries of thyroidectomized rats appear to show, at least qualitatively, the same type of response to estrin as has been observed in normal animals, but the adrenals do not show the increased size which usually is found in estrin-treated rats.

Respiratory effects of brain stem transections. HAYDEN C. NICHOLSON and JUAY HONG (by invitation). *Dept. of Physiology, Univ. of Michigan.* In dogs anesthetized with morphine-urethane, pentobarbital or ether section through the middle or upper pons commonly resulted in respiratory arrest and death. This was not true of unanesthetized, decerebrate dogs. In these animals mid-pontine transection and vagal block was followed by a deep inspiration which might be held from 15 seconds to 4 minutes. Superimposed upon this maintained inspiration there occurred rhythmic respirations at a normal frequency (usually 15 to 20 per min.). The amplitude of these respirations might vary from 1/20 to ½ the amplitude of the deep inspiration upon which they were superimposed. Following one of these relatively shallow respirations the maintained inspiration gave way. The duration of the subsequent expiration varied from 10 seconds to a minute or more and was followed by another deep inspiration. With an increase in the time following transection there was a tendency toward a decrease in the duration of the inspirations. There was, in general an inverse relationship between the durations of inspiration and expiration. This type of breathing has been observed for several hours following the section. Thus, respiration in the decerebrate, vagotomized dog following mid-pontine transection was rhythmic in respect both to the periods of maintained inspiration and to the respirations superimposed upon that inspiration.

Section through the upper medulla, with vagi intact or sectioned was followed by rhythmic breathing quite normal in type and frequency.

Relation of renin response and tachyphylaxis in acute renal hypertension. ERIC OGDEN, ERNEST W. PAGE (by invitation) and G. J. HILDEBRAND (by invitation). *Division of Physiology, Univ. of California Medical School, Berkeley.* A clamp producing complete closure of the aorta above the renal arteries produces in all (18) anesthetized cats and in most dogs a rise of carotid blood pressure having two components.

The first rise is immediate, inconstant (0–90 mm. Hg) and due almost certainly to the mechanical effect obstructing the cardiac output. The second starting in a few minutes achieves a maximum (25–90 mm. Hg) in about half an hour. This latter rise is demonstrably dependent upon the passage of blood through the kidneys via

anastomoses around the part of obstruction particularly by way of the external mammary and epigastric system.

The effect is not altered by stripping the renal pedicles and painting them with alcohol: but it is abolished by nephrectomy or clamping of the renal pedicles.

This rise may be produced and abolished repeatedly by applying and removing the clamp to the aorta. After such repeated rises, cats were made tachyphylactic to renin and then failed to demonstrate the delayed rise of blood pressure on clamping the aorta. In six experiments it was observed that the response to a standard dose of renin is strongly diminished during the hypertensive phase of these experiments; but with the disappearance of the hypertension after the full blood flow through the kidneys has been reestablished, the renin response returns and grows progressively larger.

A blood pressure raising principle in thermal shock. Wm. H. Olson (by invitation) and H. Necheles. In numerous experiments on burns, we were struck by the observation that blood pressure usually rose during the burning and, in most animals, remained at control levels or higher for five to six hours after the thermal trauma. Various determinations indicated the presence of a severe degree of shock. In experiments on traumatic shock, the contrary was observed; namely, low pressures following trauma.

Injections of small amounts of blood from burned animals into cats or small dogs showed a slight but constant increase of blood pressure in the test animal, and also seemed to sustain and even elevate the blood pressure of the test animal.

In order to analyze this phenomenon, the blood pressure response of burned animals to small doses of acetyl choline was tested, using a method previously described. (A. J. Physiol. **124:** 142, 1938) This response was found greatly reduced following burns, and it is tentatively proposed that reflex liberation of a vasopressor substance from the posterior pituitary gland plays a rôle in maintaining blood pressure following burns.

The blood pressure in most burned animals was rather high during the entire experiment; it dropped terminally, and then precipitously. We feel therefore, that blood pressure readings in the burned human may be misleading, and that other determinations like circulating time, blood CO_2, and hemoconcentration should be relied upon to indicate the degree of shock.

Cortical pre- and postmotor foci influencing the gastrointestinal tract and their efferent pathways. M. J. Oppenheimer (by invitation), E. A. Spiegel, K. Weston (by invitation). *Depts. of Physiology and of Experimental Neurology. Temple Univ. School of Medicine, Philadelphia, Pa.* In the dog's brain the gastrointestinal tract may be influenced not only from the premotor area (Brodmann's area 6) as demonstrated by Fulton and his school, but also from a second focus in a "postmotor" region (areas 5 or 3 respectively). Reactions of the gastrointestinal tract may be obtained in some animals only from one of these areas (either the premotor or the postmotor), in others this system can be influenced from both foci. Simultaneous records of the stomach, small intestine and colon showed that the whole gastrointestinal tract may receive impulses from the pre- as well as from the postmotor area. The effect may be synergic in all parts of the gastrointestinal system, or increase of activity in one part (e.g., small intestine) associated with inhibition in other parts (e.g., stomach and colon) of the gastrointestinal system. Sometimes, however, the effect is restricted to a limited part of this system. The variability of the effects obtained by stimulation of a certain focus seems at least partly due to peripheral factors, such as the degree of distension of the smooth muscle. In a second series of experiments circumscribed chronic lesions were placed in the postmotor area and the descending pathways traced by the Marchi method through the cerebral peduncle into the pyramidal tract.

The action of cyclopropane on the automatic tissues of the heart. O. S. Orth (by invitation), W. Vernon Lee (by invitation) and Walter J. Meek. *Dept. of Physiology, Univ. of Wisconsin Medical School, Madison.* (Read by title.) High concentrations of the anesthetic were administered to dogs by connecting them through an endotracheal tube and carbon dioxide absorbing canister to a small rubber bag which could be filled from a large reservoir containing a cyclopropane-oxygen mixture of known composition. Controlled respiratory exchange was carried out by manual pressure on the small bag. Cardiac irregularities were determined by Lead II electrocardiograms. Blood samples were drawn at the height of the irregularities.

Six animals were anesthetized in eight experiments with 40 per cent cyclopropane in the cyclopropane-oxygen mixture. Ventricular extra-systoles occurred twice. Fifteen animals received 50 per cent cyclopropane. Irregularities occurred in ten of sixteen experiments. Twelve animals received 60 per cent cyclopropane. In fifteen experiments on these animals idioventricular rhythms or ventricular tachycardias were seen twelve times. Twelve animals received 70 per cent cyclo and 30 per cent oxygen. In each of fifteen experiments serious irregularities were noted. Ventricular fibrillation caused death in four cases, and idioventricular rhythm progressed to cardiac standstill in another.

Blood analyses showed more than 90 per cent oxyhemoglobin always present, and in most cases the hemoglobin saturation was approximately 100 per cent. The cardiac irregularities were therefore not of anoxemic origin. The incidence of the irregularities increases as the percentage of cyclopropane in the mixture is raised.

The comparative effects of inositol and lipocaic in depancreatized dogs. FREDERICK M. OWENS, JR. (by invitation), J. GARROTT ALLEN (by invitation), DOROTHY STINGER (by invitation) and LESTER R. DRAGSTEDT. *Dept. of Surgery, Univ. of Chicago, Chicago, Ill.* The reported lipotrophic activity of inositol and the possibility that small amounts of this substance may be present in some preparations of lipocaic suggests that the physiologic activity of this hormone might be due to inositol. Comparison of the effectiveness of inositol and lipocaic in curing the fatty livers of depancreatized dogs was made. Although free inositol was observed to exert some lipotrophic activity it did not preserve life and its lipotrophic effect was not as marked as that secured by an identical amount of a preparation of lipocaic. Moreover inositol exerted little or no effect on other symptoms of lipocaic deficiency. Insulin tolerance was increased slightly or not at all, glucose excretion was little elevated, and there was no preservation of body fats such as may be secured by lipocaic.

Electroencephalographic studies on monkeys with chronic Jacksonian seizures. B. L. PACELLA (by invitation), S. E. BARRERA and L. M. KOPELOFF (by invitation). *Depts. of Psychiatry and Bacteriology of the New York State Psychiatric Inst. and Hospital, New York City.* Preliminary electroencephalographic observations were made on a series of 25 rhesus monkeys exhibiting convulsive manifestations of the unilateral Jacksonian or generalized type. These convulsive movements occurred spontaneously about 4 to 6 weeks following the application of various chemical and immunologic preparations to the precentral area ("motor") of the cerebral cortex on one or both sides of the brain. Untreated monkeys as well as animals treated with "indifferent" substances served as controls. Electroencephalograms were taken at different intervals following the surgical procedure, and were recorded by means of a two-channel, push-pull amplifying system with ink-writers attached. The animals were anesthetized by means of sodium pentobarbital (0.0125 g per kilo), half the dose given intravenously and the remainder intramuscularly. A total dose of 0.05 to 0.075 was usually sufficient to produce a mild narcosis from which the animal could be easily roused. Six small solder electrodes were attached to the scalp over the corresponding opposite prefrontal, motor and occipital areas. Simultaneous recordings were made from each side of the head employing various combinations of the electrodes. Subsequently, 1–2 cm^3 of metrazol was injected intramuscularly and the recordings repeated twenty to thirty minutes later.

The data revealed a significant correlation between the clinical manifestations and the electroencephalographic findings. Where unilateral seizures were observed abnormal electrocortical activity was most pronounced over the corresponding contralateral motor area. However, bilateral electroencephalographic abnormalities were demonstrated in animals manifesting generalized convulsive seizures.

The effects of vasoconstriction on the apparent viscosity of blood flowing through the hindlimb of the dog. J. R. PAPPENHEIMER and J. P. MAES (Fellow of the Belgian American Educational Foundation) (introduced by Magnus I. Gregersen). *Dept. of Physiology, College of Physicians and Surgeons, Columbia Univ., New York City.* The relation between pressure and flow of blood in the isolated perfused hindlimb of the dog was found by Whittaker and Winton (J. Physiol. **78**: 339, 1933) to be approximately linear except at low pressures. Extrapolation of the linear region of the pressure-flow curve was found to intersect the pressure axis at about 20 mm. Hg. However, this value does not represent the minimum pressure required to obtain a flow of blood, for at low pressures the slope diminishes rapidly and the curve approaches the origin.

During vasoconstriction the pressure at which this deviation from linearity occurs is increased and the extrapolated intercept is greater. No such deviations occur when Ringer's solution is substituted for blood and the ratio of Ringer flow to blood flow at constant pressure (apparent viscosity) is increased during vasoconstriction. The phenomenon has been investigated in innervated perfused limbs in which vasoconstriction results from the vasomotor tone and in isolated preparations during infusion of adrenalin. An increased resistance to blood flow due to vasoconstriction consists of two components: *a*, a linear increase of resistance; *b*, a non-linear increase in the internal resistance (apparent viscosity) of the blood. A method for separately evaluating the two quantities will be presented and the application of the method to the quantitative measurement of the vasomotor tone will be discussed.

Action of anterior pituitary growth hormone on protein metabolism of rats poisoned with monoiodoacetic acid. KARL E. PASCHKIS (J. Ewing Mears Fellow) (introduced by J. Earl Thomas). *Depts. of Physiology and Medicine, Jefferson Medical College of Philadelphia.* Certain extracts of the anterior pituitary gland enhance protein

anabolism. This effect has been produced by growth hormone preparations both in normal and in phlorrhinized animals. Experiments were performed to study the effect of growth hormone preparations (Antuitrin G) in animals with abnormal protein metabolism. White rats were treated with moniodoacetic acid and the effect of the pituitary extract on Urea N, Amino N and Sugar of the blood was studied. In the normal rats Urea N content and Amino N content of the blood decrease 6 hours following injection of the extract. In rats treated with monoiodoacetic acid the Urea N content increased, whereas the Amino N content decreased. These changes were not influenced by anterior pituitary extract. Monoiodoacetic acid has been shown to interfere with normal growth by drawing on S- containing amino acids. On the other hand anterior pituitary extract has been shown to produce S retention in the normal dog, thereby indicating that its protein anabolizing action includes S- containing protein constituents. The fasting blood sugar was normal in rats treated with monoidoacetic acid. Following administration of the ant. pit. extract, the blood sugar decreased in one third of the animals. It has been shown previously that the hypoglycemia following injection of Antuitrin G is not linked to the changes of Urea N in either the normal, the adrenalectomized and the pancreatectomized rat. This is also true for iodoacetic acid treated rats. The experiments reported here suggest that anterior pituitary extract does not exert its protein anabolizing action in rats whose protein metabolism is abnormal due to monoiodoacetic acid poisoning. Hypoglycemia due to the anterior pituitary extract is observed in one third of the animals.

The simultaneous gastric and duodenal motility phases in man. T. L. PATTERSON and D. J. SANDWEISS (by invitation). *Dept. of Physiology, Wayne Univ. College of Medicine and Harper Hospital, Detroit, Mich.* This study is an attempt to correlate synchronously gastric motility and duodenal motility phases with the normal hunger sensation in man. The dual balloon-kymograph-bromoform manometer method was employed. Fluoroscopic examination was used to determine the location of the tubes and balloons which procedure was aided by inserting a metal tip into the end of a special Miller-Abbott tube at which point the duodenal balloon was attached. An electric signaling device operated by the subject recorded the normal hunger sensations.

The results of these motility studies may be briefly summarized as follows: 1. Gastric motility may occur simultaneously with duodenal motility. 2. Duodenal motility may occur in the absence of gastric motility (quiescent stomach). 3. Some evidence exists that both the stomach and duodenum may be completely quiescent at times. 4. There is a possibility that gastric motility may exist in the absence of duodenal motility (quiescent duodenum).

Hunger sensations will be discussed in relation to the above motility phases.

The ketonic steroids of pregnancy urine. W. H. PEARLMAN (by invitation) and GREGORY PINCUS *Physiological Labs., Clark Univ., Worcester, Mass.* Allopregnanol-3(β)-one(20), previously reported in pregnancy urines of sows and mares (Marker et al), is present also in human pregnancy urine obtained during labor and delivery. Pregnanol-3(α)-one(20), m.p. 148°, was also isolated, thus confirming a previous report (Marker et al) of its occurrence in human pregnancy urine. Approximately 0.05 mgm. and 0.3 mgm. of the respective steroids were isolated per liter of labor and delivery urine.

The excretion of C-17 and C-20 ketosteroids during pregnancy can be followed with the aid of the Zimmerman reaction. When known mixtures are employed consisting of pure dehydroisoandrosterone and pregnenolone, representative respectively of the above ketonic types, it is possible to determine simultaneously either component with an accuracy of $\pm$ 10 per cent in this test. The marked differences in the absorption spectra permit one to make the necessary calculations. The Zimmerman test can thereby be useful not only in studies of androgen excretion but also in studies of progesterone metabolism. However, indiscriminate application of the Zimmerman test in estimating 17-ketosteroids in pregnancy urine will lead to gross error and, indeed, such errors have already appeared in endocrinological literature. Certain aspects of progesterone metabolism will be discussed in the light of the foregoing observations.

The humoral nature of the factor causing the let down of milk. W. E. PETERSEN and T. M. LUDWICK (introduced by M. B. Visscher). *Division of Dairy Husbandry, Univ. of Minnesota.* Ely and Petersen (J. Dairy Science **24**: 211, 1941) demonstrated that the oxytocic principle is pharmacologically active in the let down of milk in the bovine as intravenous injection of this hormone forced the milk out of the alveoli. While they postulated that the milking act, reflexly, caused the ejection of the hormone from the posterior pituitary into the blood stream they did not prove this point.

Evidence is presented that the factor causing the let down of milk is humoral in nature. Surviving bovine mammary glands, perfused with blood from the donor, were cannulated to drain them of all the milk that would drain out. After the milk ceased flowing and with the cannulae in situ a liter of blood drawn from cows that had been

stimulated to let down their milk was introduced as the perfusate. Within 15 seconds after the introduction of such blood the milk flowed copiously out of the cannulae. Blood from cows that were not stimulated to let down their milk had no effect. Blood from cows that were markedly excited did not cause a let down of milk but had pronounced vaso-constricting properties reducing the blood flow to as little as one fourth normal.

When the blood was permitted to stand for one half half hour the humoral agent for the let down of milk was destroyed. In in-vivo experiments it was found that the intravenous injection of adrenalin interfered with the let down of milk even when the injections were made after the cows had been stimulated to let down their milk. Fright or excitement had similar effects to those of injected adrenalin. [*Scientific Journal Series Paper no. —, Minnesota Agricultural Experiment Station. Prepared with the assistance of the Work Projects Administration, Official Project no.* 665-71-3-69. *Sponsor: University of Minnesota.*]

Toxicity of paraldehyde for both young and old rats. G. W. PHILLIPS (by invitation), EMMETT B. CARMICHAEL and F. A. KAY (by invitation). *Dept. of Physiological Chemistry, School of Medicine, Univ. of Alabama, University, and Bryce Hospital, Tuscaloosa.* (Read by title.) Normal young and adult rats were used in this series of experiments which extended over four months, September to December inclusive. The animals were arbitrarily divided into two weight groups: 100 to 199 grams and 200 to 525 grams. There were 57 of the former group that weighed from 105 grams to 197 grams and 107 of the latter group that weighed from 205 grams to 525 grams.

The paraldehyde was diluted with nine volumes of water and injected intraperitoneally. The doses varied by increments of 0.25 cc. from 12 cc. to 15 cc./kilo. The preliminary results indicate that the average lethal dose for the smaller rats is about 13.75 cc. to 14.5 cc./kilo and for the larger rats is about 13.5 cc. to 13.75 cc. of the 1:10 solution of paraldehyde/kilo.

The source of the ammonia produced in the kidney in acidosis. R. A. PHILLIPS, P. H. FUTCHER (by invitation), P. B. HAMILTON (by invitation), R. M. ARCHIBALD (by invitation), A. E. HILLER (by invitation) and D. D. VAN SLYKE. *Hospital of The Rockefeller Inst., New York City.* The neutralization of acid by the kidney is, in the presence of a severe acidosis, accomplished in part by the formation of ammonia. This formation of ammonia permits the organism to conserve its base, since ammonia must come from some nitrogenous source. From theoretical considerations and indirect experimental procedures both amino-acids and urea have been considered the most likely sources. These experiments are a direct attack upon the problem.

Several dogs have had one kidney removed and the other one explanted beneath the skin, so that blood can be readily obtained from the renal vein. The carotid arteries were likewise transplanted beneath the skin for ready sampling. Acidosis was produced by administering HCl to these animals when they were on a low-protein diet consisting of uncooked starch and cream. In the absence of anesthesia, in these well-trained dogs, a catheter is inserted in the bladder and samples of urine were collected at 10–20 minute intervals. At the mid-point of the urine periods, samples of carotid arterial blood and renal venous blood were obtained. The blood and urine samples were analyzed for urea, creatinine, amino-acids and ammonia. The renal blood flows were obtained from the creatinine values. The rates of removal from the blood were compared with the rates of excretion of ammonia, urea, and amino-acids. In experiments in which the rate of ammonia excretion per minute was greater than the rate of urea excretion the source of ammonia appears to be the amino-acids.

Nutrition of the rabbit ovum in vitro. GREGORY PINCUS. *Physiological Lab., Clark Univ., Worcester, Mass.* The slow growth of rabbit blastocysts in rabbit serum *in vitro* is not accelerated by the addition of pantothenic acid, riboflavine, nicotinic acid amide, or coenzyme II. Thiamine in catalytic concentrations markedly stimulates blastocyst growth and enhances the moderate growth stimulation provided by pyruvate. On the assumption that the process of growth stimulation involves the metabolism of pyruvate in a system requiring thiamine various possible pyruvate systems were tested. Pyruvate transamination seems unlikely since alanine in various combinations proved ineffective as a growth stimulating substance. Oxalacetic and α-ketoglutaric acids were ineffective. Acetaldehyde did not enhance growth and failed to facilitate the pyruvate effect.

Artificial media containing the salt, protein, glucose and amino acid concentrations found in mammalian body fluids did not support growth at all. The addition of thiamine to such media led to a slight spurt of growth. This growth spurt is attributed to the utilization of endogenous substances catalyzed by thiamine. The usefulness will be indicated of the methods employed as a means of determining the nutritive requirements of mammalian ova.

The site of mechanisms determining frequency and recruitment of phrenic neurones. R. F. PITTS. *Dept. of Physiology, New York Univ. College of Medicine, New York City.* An attempt to analyze the respiratory neural mechanisms responsible

for increased frequency of discharge and recruitment of additional neurones in response to carbon dioxide inhalation (Adrian and Bronk; Bronk and Ferguson; Gesell, Atkinson and Brown) has been made by observing the response of phrenic motor neurones of the cat during successive elimination of known elements of the respiratory complex.

Transection of the brainstem at various levels from the rostral end of the midbrain to the caudal end of the pons was without any significant effect upon the character of the response to carbon dioxide inhalation. Transection at lower levels led to immediate death. In animals with the brainstem transected at the collicular level, bilateral vagal section led to characteristic slowing of respiration. Response to carbon dioxide inhalation was normal in so far as increased frequency of discharge and recruitment occurred but respiratory rate failed to increase. In animals with the brainstem transected through the middle of the pons or lower, bilateral vagal section was followed by maintained phrenic neurone discharge (inspiratory cramp or apneusis of Marckwald, Lumsden, Stella, etc.). Frequency of discharge and numbers of neurones active increased with accumulation of carbon dioxide, much as in the normal animal although rhythmic respiration was abolished. It is concluded that the neural mechanisms determining the frequency of inspiratory motor neurone discharge and numbers of neurones active are resident within the lower part of the medulla oblongata and are independent of reflex connections.

The disk-sphere transformation of red cells by lecithin. ERIC PONDER. *The Nassau Hospital, Mineola, L. I., N. Y.* The disk-sphere transformation of mammalian red cells produced by lecithin can be studied quantitatively if (a) lecithin is added as an isotonic sol in saline and (b) plastic slides and cover-glasses are used instead of glass ones.

A study of the quantity of lecithin needed to produce sphering shows that 1, the amount is about enough for monolayer formation at the cell surface; 2, the temperature coefficient is positive; 3, formol-fixed cells require increased quantity for transformation; 4, neither osmotic pressure change nor sub-lytic quantities of lysins affect the phenomena. The action of lecithin suggests that a pre-existing expansive surface force is being acted against and overcome, as in an elastic or plastic system.

Spherical forms produced by lecithin can be promptly transformed to disks by adding benzene, ether, chloroform, or other solvents with lipoid affinity.

Ghosts cannot be sphered even with large quantities of lecithin. The surface structure apparently has a "permanent set." Poikilocytes can be sphered, the major projections rounding off slowly and incompletely. The transformation of reticulocytes into spheres is restrained by the reticulum.

Taken with the facts already known about disk-sphere transformations, these observations lead to the idea of the red cell surface as a liquid crystal, with the properties of an expanded and elastic sheet.

The acute effects of peripheral venous occlusion on blood flow in the dog's leg. W. H. PRITCHARD, (by invitation) A. S. WEISBERGER (by invitation), E. F. SCHROEDER (by invitation), R. E. SHIPLEY (by invitation) and D. E. GREGG. *Dept. of Medicine, Western Reserve Univ., School of Medicine, Cleveland, O.* Mean blood flows in the femoral artery and vein have been simultaneously measured with appropriate rotameters in dogs under various anesthetics. Normal phasic respiratory flow fluctuations occurred; during inspiration venous flow increased and arterial flow decreased. Presumably, these changes were passively induced by respiratory changes in blood pressure.

During acute femoral vein occlusion, as the venous pressure rose, the arterial flow at first increased considerably and then later rose somewhat, but never regained the control value. Upon vein release, both flows were briefly augmented before returning to pre-occlusion levels. During femoral artery occlusion, similar flow changes occurred in the femoral vein. In neither case did the flow increase following vessel release equal the flow deficit during the occlusion period.

Using the thermostromuhr, Linton et al (Am. Heart J. **21:** 721, 1941) found that peripheral venous occlusion increased arterial inflow. We believe this apparent flow increase, in this instance, results from the inability of the thermostromuhr to indicate correctly the directional change in flow. Investigation shows (1) that peripheral venous occlusion or the application of a tourniquet induces or augments back flow in the femoral arterial flow pattern, (2) such increase in back flow is recorded by the thermostromuhr as an increase in flow, whereas actually the flow is reduced. [*Supported by a grant from the Commonwealth Fund.*]

The influence of hydrochloric acid on the motor activities of the pyloric sphincter region and on the process of gastric evacuation. J. P. QUIGLEY, M. R. READ (by invitation), K. H. RADZOW (by invitation), I. MESCHAN (by invitation) and J. M. WERLE (by invitation). *Dept. of Physiology, Western Reserve Univ. Medical School, Cleveland, O.* By direct observations employing the balloon-water manometer technic in trained dogs we demonstrated that 5–10 cc. of 0.2–0.4 per cent HCl introduced into the distal duodenum inhibited the pyloric antrum, sphincter and duo-

denal bulb. Studies by the fluoroscopic-optical manometer technic under the same conditions likewise showed inhibition of the entire sphincter region. The inhibitory effect of HCl was less than comparable quantities of fats or fatty acids. When the HCl was injected into the extreme proximal duodenum a transient augmentation of sphincteric and bulbar activity during the injection period preceded the inhibition of these structures. This augmentation was much less marked in fed than in fasting animals.

Gastric evacuation temporarily ceased during the intraduodenal administration of HCl. This cessation in evacuation resulted primarily from the inhibition of antral peristalsis and the concomitant absence of positive antral-bulbar phasic pressure gradient waves. The changes in sphincter and bulbar activity played only a minor role; even when this activity was exaggerated it was of the type which would not prevent evacuation but would hasten aboral propulsion of irritant material from the bulbar region. [*This investigation was supported by a research grant from the Ella Sachs Plotz Foundation.*]

Observations on the metabolism of human fetal brain in vitro. E. RACKER (introduced by Herman Kabat). *Cardiff City Mental Hospital, Cardiff, Wales, England.* (Read by title.) An opportunity presented itself to study the respiration of two human fetal brains in the Warburg manometric apparatus, very shortly after death. Fetus I was 6 months old and fetus II 5 months old. Oxygen uptake of slices of cerebral cortex were measured within 60 minutes after death in the case of fetus I and within less than 50 minutes in the case of fetus II. The slices respired in a Locke phosphate glucose medium at 37°C. in oxygen. The experiments were done in duplicate. The Q_{O_2} over the first hour for cortex slices was 5.9 in I and 6.4 in II. The respiration of slices of medulla oblongata was studied in fetus II and a Q_{O_2} of 8.8 was observed. The Q_{O_2} of human fetal cerebral cortex slices appears to be lower than that of cortex slices of adult rats, cats, and dogs. Similar results have been recorded for infant dog brain by Himwich and his co-workers.

The oxygen uptake of human fetal brain slices was not inhibited by 0.1 per cent morphine hydrochloride. Inhibition of oxygen uptake of 20 per cent was observed with 0.25 per cent morphine on minced human fetal brain, which is similar to results obtained with adult mammalian brains. Since the above concentrations of morphine are much higher than those effective in vivo, the toxic effect of morphine cannot be related to a general decrease of brain respiration.

The demonstration of conjugated estrogens in human pregnancy serum, especially in the protein fraction. A. E. RAKOFF (by invitation), K. PASCHKIS (J. Ewing Mears Fellow) (by invitation) and A. CANTAROW. *Depts. of Obstetrics, Physiology and Medicine, Jefferson Medical College.* Serum from the blood of pregnant women was hydrolyzed by acidifying to pH 1.0 and heating in a water bath for 6 hours. The gelatinous mass was extracted with ether-alcohol. The extract was dried, taken up in corn oil, and assayed biologically for estrogenic activity, while the remaining precipitate was dried, suspended in water, and assayed similarly. Estrogens were demonstrated in both fractions, the greater proportion being in the ether-alcohol extract. Their combined estrogenic activity was significantly greater than the free estrogen content of the untreated serum as determined by direct biologic assay. These observations suggest that human pregnancy serum contains conjugated (?) estrogen and that some of the estrogen may be "bound" to the protein. In order to study further the latter possibility, the protein fraction was separated by 1, salting out with sodium sulfate; 2, precipitation with sulfosalicylic acid, and 3, ultrafiltration. In each instance the protein-free filtrate exhibited little or no estrogenic activity. From the protein fraction, only a part of the expected estrogen content could be removed with ether-alcohol, even after hydrolysis. Some estrogenic activity was present in suspension of the remainder. These observations suggest that the estrogens of pregnancy serum do not pass through a collodion membrane, that they are precipitated with the protein fraction and that they cannot be removed completely from the latter by ether-alcohol extraction.

The influence of adrenalectomy in black rats on normal and defficient diets, with special reference to pigmentation of the coat and hair follicles. ELAINE P. RALLI and IRVING GRAEF. *New York Univ. College of Medicine, New York City.* In continued observations on rats deficient in the filtrate-fraction of Vitamin B, the effects of adrenalectomy were noted. 55 black or brown rats have been adrenalectomized and followed up to 9 weeks. 18 were on complete and 37 were on diets deficient in the filtrate-factors, prior to adrenalectomy. The periods of the deficient diets varied from 24–110 days. Development of greying varied with the duration of deficiency. After adrenalectomy para-aminobenzoic acid, pantothenic acid, or the complete filtrate-fraction was added to the deficient diets of 13 rats. Of the 18 rats on complete diets, 9 were given filtrate-factor deficient diets following adrenalectomy. All rats received 1 per cent NaCl solution. At operation the dorsal fur was shaved. The shaved skin had a pinkish color. In all animals about 7 days after adrenalectomy, the skin developed a deep bluish hue. This increased and extended over most of the torso. The fur that regrew over the shaved areas was

deep glossy black or brown depending on its original color, and was abundant. Pigmentation increased progressively in adrenalectomized animals on complete diets. In adrenalectomized rats on deficient diets, the skin paled and greying of the glossy black fur appeared after varying intervals, apparently depending on the type and duration of the diet prior to adrenalectomy. Butcher reported luxurious growth of fur in adrenalectomized albino rats.

Preliminary studies of shaved skin show an excess of pigment granules in follicles, roots and shafts of newly forming hairs. The overlying cutis thus far studied shows no increased pigment storage.

Rats fed complete diets after adrenalectomy survived longer than rats on deficient diets. [*This investigation was aided by a grant from the Josiah Macy Jr. Foundation.*]

Absence of fatigue of the contractile mechanism in single muscle fibers. ROBERT W. RAMSEY and SIBYL F. STREET (by invitation). *Physiology Dept., Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.* Fatigue of a whole muscle, in the sense that the word is usually used, implies that each individual muscle fiber in the muscle exerts less force than it would exert if unfatigued and that the decrease of force is graded with the extent of fatigue. In single muscle fibers, however, the usual result of a continuous tetanic stimulation is that the initial tension is maintained fully for some time, and then decreases abruptly to zero as a consequence of failure of the maximal or supermaximal stimulus to excite. Under certain circumstances there is a partial fall of tension to a fixed level in a tetanus of single muscle fibers followed by an eventual abrupt decrease to zero as in the above. We believe that this partial fall is not an expression of fatigue, but is due to other factors which will be discussed. From the results as a whole we are led to infer that possibly there is no fatigue of the contractile mechanism in the usual sense of the word and that the graded "fatigue" observed in whole muscle is due to the successive decrease of the numbers of individual fibers.

Studies on absorption from the rumen. A. D. RANKIN (introduced by H. H. Dukes). *Dept. of Physiology, New York State Veterinary College, Cornell Univ., Ithaca.* (Read by title.) This is a report of preliminary work. The experiments were performed on four animals, two of them with permanent rumen fistulas and two with temporary fistulas.

Most of the experiments concerned the absorption of dextrose. In ten experiments with two sheep having permanent rumen fistulas, dextrose solution was placed in the posterior ventral sac of the rumen. The dextrose was administered in the ratio of four grams per kilogram, and in approximately 2 M solution. Enough methylene blue was added to give the solution an intense blue color so that the efficacy of the localization of the solution could be determined. A marked rise in the blood sugar level always resulted. The increases over the level at the time of administration varied from 34 per cent to more than 400 per cent. Following is a typical series of blood sugar values as determined every 15 minutes: pre-administration—67 mgm. per 100 cc., 65, 62, 64; post-administration—79, 98, 109. Control experiments in which water was substituted for the dextrose solution showed no significant change in the blood sugar level.

These findings are contrary to those of Aggazzotti. They confirm by a better technic the results of Wester.

Other experiments revealed that potassium iodide, pilocarpine, strychnine, and sodium cyanide can all pass through the mucosa of the rumen. The pilocarpine experiments confirm those of Trautmann.

These experiments lend strong support to the growing belief that the rumen is an organ of absorption.

A comparative study of normal, rachitic and healed rachitic bone by x-ray diffraction technic. C. I. REED, H. C. STRUCK and B. P. REED (by invitation). *Univ. of Illinois, Chicago Colleges.* Study of tibial cortical sections of normal bone shows a preferred orientation of calcium salts along the axis of the shaft. In rickets there is loss of preferred orientation, and disorganization of the protein matrix. Healing does not completely eradicate the rachitic pattern even after 275 days, although roentgenograms and tensile strength tests show complete recovery. Further study is directed toward an interpretation of the biological significance of these findings.

Comparative studies on different bones of the same animal show characteristic patterns for each type of bone. So far as this technic is concerned it appears that flat bones are less affected than long bones and vertebrae.

The effect of variations in external resistance on the electrical activity of the stomach. W. S. REHM (introduced by Hampden Lawson). *Dept. of Physiology, Univ. of Louisville School of Medicine, Louisville, Ky.* Low resistance non-polarizable Zn-$ZnSO_4$ electrodes with an outer layer of mammalian Ringer-agar were placed opposite each other, one in contact with the serosa, the other in contact with the mucosa on the fundic portion of the stomach in barbitalized (250 mgm. per kgm.) or pernoctinized (40 to 60 mgm. per kgm.) dogs. Under these conditions the open circuit voltage measured by a potentiometer was comparatively stable, often varying less than 1

mv. in 10 minutes. The range of P.D. was from 26 mv. to 71.5 mv. with the serosa positive to the mucosa in the external circuit. The electrodes were connected in series with a variable resistance box and the current was determined from the measured IR drop across the known resistances.

The terminal voltage (IR drop through circuit minus IR drop through stomach) plotted against the current in the external circuit gives a straight line, the slope of which is equal to the resistance of the stomach. The lowest total external resistance was 20 ohms. The resistance of the stomach varied from 110 ohms to 290 ohms per sq.cm. With an electrode area of 20 sq.cm. over 1500 microamperes could be drawn off continuously for as long as a half-hour without a steady fall in the magnitude of the current. The straight line relationship between terminal voltage and external current indicates that neither the E.M.F. nor the resistance of the stomach changes during the flow of current.

Excitation of spinal neurons by centripetal impulses in ventral root axons. BIRDSEY RENSHAW. *Labs. of The Rockefeller Inst. for Medical Research, New York City, and Oberlin College, Oberlin, O.* Centripetal volleys which enter the spinal cord over alpha fibers of ventral roots (cats; rabbits) initiate spike activity lasting 30–50 msec. in the ventral horn. Micro-electrodes make it possible to record activity of single participating neurons in effective isolation. Each neuron responds to a single centripetal volley with a repetitive discharge of as many as 15–20 spikes. The first spike has a minimum latency, measured from the time of arrival of the centripetal volley at the cord, of 0.7 ± msec. The initial frequency is often so high (over 1500/sec.) that the second and subsequent spikes are subnormal in size. The successive spikes are spaced at progressively increasing intervals.

The spikes are not repetitive centripetal discharges from the periphery. No comparable centrifugal impulses in motor axons have been detected. It is therefore inferred that the discharges represent activity of interneurons located within the ventral horn. The available evidence indicates that the discharges are not injury effects associated with the presence of the recording microelectrode.

Impulses in many ventral root axons regulate the excitation of each neuron. In general, the larger a centripetal volley in a deafferented nerve or ventral root, the more prolonged are the discharges of excited neurons and the greater their frequencies. A neuron's discharge to a centripetal volley in one deafferented peripheral nerve is often conditioned (augmented or decreased) by simultaneous or preceding volleys in a second nerve. Dorsal root volleys, except as they result in the firing of motoneurons, have little effect.

Potentiation of epinephrine toxicity by sodium bisulfite. R. KOHN RICHARDS. *Abbott Labs., North Chicago, Ill.* Epinephrine HCl solution 1:1000 prepared according to the U.S.P. was found to have an LD50 of approximately 5 mg. per kg. subcutaneously and 3.5 mg. per kg. intramuscularly in rats. Sodium bisulfite as a stabilizing agent is permitted by the U.S.P. to be added in concentrations up to 0.5 per cent, and most of the commercial solutions contain 0.1 to 0.2 per cent sodium bisulfite. It was found that the addition of 0.2 per cent of this stabilizing agent increases the toxicity of the epinephrine to 1.0 mgm/kgm. subcutaneously and 0.4 mgm/kgm. intramuscularly. Sodium bisulfite in itself is tolerated in doses up to 300 mgm/kgm. in rats. A similar increase of epinephrine toxicity by the addition of sodium bisulfite has been found in mice. Since the intravenous lethal dose is not significantly influenced by the addition of sodium bisulfite, the marked increase of toxicity must be due to a local effect, possibly the speed of absorption. A number of other acidifiers and reducing agents added to the epinephrine instead of the sodium bisulfite were found to possess no or only very slight potentiating effect upon epinephrine toxicity. Further experiments designed to elucidate the mechanism of action are being conducted.

Toxicity and tolerance of para-amino benzoic acid. R. KOHN RICHARDS. *Abott Labs., North Chicago, Ill.* (Read by title.) Recently, it has been reported that para-aminobenzoic acid can restore gray hair to its original color if fed to humans or rats in very large doses. It was, therefore, of interest, to determine the tolerance of the administrations of large amounts of this material. In our experiments para-aminobenzoic acid solubilized with sodium hydroxide at a pH of approximately 6.0 was used in rats. Intravenously, only 30 per cent of the animals died after injection of 4 gm./kgm. The symptoms consisted of convulsions and respiratory paralysis and might also be due to the high osmotic pressure of the solution. Intraperitoneally, 40 per cent died on a dose of 4 gm./kgm. within a few hours. Orally, no deaths occurred unless at least 6 gm./kgm. were fed daily for 3 successive days. The LD50 in rabbits is approximately 2 gm./kgm. intravenously. If young rats were injected with doses of 200–500 mgm/kgm. daily for 21 days their growth rate was slightly better than that of the controls. Urine and blood status showed no abnormalities. The blood pressure of dogs and cats was slightly raised by doses of 100 mgm/kgm., but this raise depended on the speed of injection. No change of the respiration occurred. The oxygen consumption of rats

was slightly but not consistently increased after intraperitoneal injection of 600 mgm/kgm. of para-aminobenzoic acid.

These results seem to indicate that para-aminobenzoic acid possesses a low acute and chronic toxicity.

Gross differences in response of the liver to first and later daily dosage with insulin and prolactin. OSCAR RIDDLE and DAVID F. OPDYKE (by invitation). *Carnegie Inst., Dept. of Genetics, Cold Spring Harbor, N. Y.* Pigeons survive huge doses of insulin and their livers are known to double their weight after 4 to 7 daily injections with prolactin. Young pigeons were given from 1 to 5 daily injections of insulin or prolactin and differences between the response to a first and to subsequent injections were measured in the case of liver glycogen and fat, ketonemia and glycemia. All values were obtained at the end of a 24-hour fast. A first treatment with insulin (70 units per kilo) greatly increases (160 per cent) liver fat but later dosage rapidly reduces the 24-hour excess to or below the normal value. First, but not later, insulin injections produce ketonemia (10 hrs. after injection); twelve hours after a first injection blood sugar is decreased by 25 per cent, but 12 hours after a second daily injection it is increased by 50 per cent in both normal and hypophysectomized birds. Twenty-four hours after a first injection of prolactin the percentage of liver glycogen is increased, but third and later injections neither maintain nor produce this increase; total liver fat is increased (during 6 days) essentially parallel with increase of hepatic tissue.

Though the nutritive state of these animals is affected by temporary and prolonged dosage with either substance these results mainly reflect the ability of the first dose of these hormones to set a new level of functioning in one or another regulatory organ (adrenal, pancreas).

This investigation was supported by a grant from the Committee on Research in Endocrinology, National Research Council.

The effect of environmental temperature on thyroid function in dogs. D. S. RIGGS (Alexander Brown Coxe Memorial Fellow, Departments of Physiology and of Psychiatry and Mental Hygiene, 1940–1941) (introduced by W. T. Salter) and E. B. MAN (by invitation). *Depts. of Physiology and of Psychiatry and Mental Hygiene, Yale Univ. School of Medicine, New Haven.* Many investigations have established the essential rôle of the thyroid in protecting rodents from cold temperatures. During 5 weeks in a room at approximately 28°C, 7 healthy young dogs, receiving an adequate dietary iodine intake approximating 1.5 gamma per kilo body weight per day, had an average whole blood iodine of 0.8 gamma per cent, corresponding to a serum iodine of about 1.3 gamma per cent. When the temperature was lowered to approximately 9°C for 7 weeks the blood iodine levels remained just as low as previously. The body temperature of one female fell from 38°C to 29.5°C after 5 weeks refrigeration. Despite recovery in a warm room she died shortly after a second exposure to cold, without ever having had any increase in blood iodine. The histology of thyroid biopsies from four animals at the end of the cold period indicated only normal thyroid activity. The thyroid iodine averaged 165 milligrams per cent.

Room temperature was then maintained at approximately 21°C. Inorganic blood iodine could be elevated by giving an iodine-rich diet, yet the dialyzed serum iodine ("hormonal" iodine) of one dog on such a diet was as low as that of another on the iodine-poor diet.

The thyroid iodine at autopsy was similar to that of the previous biopsies, except that thyroids from animals receiving iodine-rich diets contained about three times as much iodine.

It was concluded that, in dogs with a normal iodine intake, blood iodine is remarkably low, and that thyroid function is but little affected by external temperature. [*Aided by a grant from the Fluid Research Fund of the Yale University School of Medicine.*]

The importance of the adrenal medulla during prolonged exposure to cold. GORDON C. RING. *Dept. of Physiology, Ohio State Univ., Columbus, O.* It is well known that the thyroid gland is stimulated by prolonged exposure to cold. The elevation in metabolism, which results from this increased thyroid activity, is small in proportion to the total amount of heat required to maintain body temperature in a cold environment. One, therefore, wonders if the most beneficial effect of the thyroid may not result from its sensitizing the calorigenic response to adrenalin. During the first two hours after injecting 0.05 cc. of adrenalin (1:500 in gelatin), control rats had a metabolic rate which was about 50 per cent above the basal level. When thyroxin was given one week before measuring the metabolic effect of adrenalin, the response was frequently doubled. Three weeks exposure to cold produced an equally great sensitization. The amount of adrenal secretion produced by the rat during exposure to cold is not known but our evidence suggests that it may be responsible for a large part of the metabolic stimulation.

Sympathomimetic and related drugs stimulating pancreatic secretion. ROBERT A. ROBACK (by invitation), HARRY GREENGARD and A. C. IVY. *Dept. of Physiology and Pharmacology, Northwestern Univ. Medical School, Chicago.* The pancreatic response to intravenous injections of a

large number of sympathomimetic drugs has been determined. Most of the compounds tested exerted an inhibitory effect on pancreatic secretion; however, several were found to be stimulators, the most potent of which were epinine (I), oxytyramine (II), and 3,4,3′4′-tetrahydroxydi (β-phenethyl) amine (III). Somewhat less potent stimulators were m-hydroxyphenethylamine (IV), 6,7 - dihydroxy - e - methyl - 3,4 - dihydroisoquinoline (V), and 6,7-dihydroxy-2,3-dimethyl-3,4-dihydroisoquinolinium chloride (VI); least potent were m-methoxy (VII)- and p-methoxyphenethylamine (VIII), 3,4-dimethoxyphenethylamine (IX), and 3,4-dihydroxyphenylalamine (X). All these compounds evidenced pressor activity with the exception of V, VI, IX, and X; no correlation was found to exist between pressor activity and pancreatic stimulation. A marked degree of structural specificity is indicated; slight modifications in chemical constitution convert a pancreatic stimulator to an inhibitor. All the potent phenethylamine derivatives were primary or secondary amines with an unsubstituted β-carbon atom and contained phenolic hydroxyls in the 3- or 3,4-positions. The type of pancreatic stimulation evidenced by these compounds differs from secretin stimulation in several respects. Both secretory and pressor responses are enhanced by cocaine administration; the pressor response is diminished or abolished by ergotamine, whereas the secretory response is unimpaired.

The effect of body size upon energy exchange in work. SID ROBINSON. *Dept. of Physiology, Indiana Univ. Medical School, Bloomington.* Two men performed work on a motor driven treadmill at rates which raised metabolism 7 to 11 times the basal level. One of the subjects weighed 99 kgm. and had 44 kgm. of weight per square meter of body surface; the other weighed 61 kgm. and had 35 kgm. per square meter. The efficiencies of the two men in performing the work were the same. In work which raised metabolism to 8 Cal./kgm. per hour when heat dissipation was limited by a room temperature of 32° C. and a humidity of 70 per cent the larger man accumulated heat throughout the work and was forced to stop with approaching heat exhaustion after 70 minutes. Under the same conditions the smaller man attained heat balance and a steady state of heart rate at a metabolic rate of 9.7 Cal./kgm. per hour. Both men in this type of work produced heat in proportion to body weight and since the ratio of weight to surface area was 20 per cent greater in the large man than in the small man his heat production in walking at a given rate when related to surface area was about 20 per cent greater. Therefore, since heat dissipation depended largely upon the skin surface, at rates of walking in which the small man could easily maintain heat balance the large man could not dissipate all of his body heat.

Studies on induced ovulation in Rana pipiens. TRUE W. ROBINSON and HENRY C. HILL, JR. (introduced by Carl G. Hartman). *Dept. of Zoology, Univ. of Illinois, Urbana.* The following method is presented for quantitative determination of the number of eggs released at any time by frog ovaries. The ovaries were excised from the frog before ovulation and placed in amphibian Ringer's solution. Excellent ovulation occurred. Counts were made periodically of the number of eggs ovulated in Ringer's and the rate and total ovulation determined. The following information was obtained.

1. When frogs which were in hibernation before shipment were kept at 12° C. after arrival, the amount of ovulation obtainable decreased after the first few days; but if they were kept at 4° C., ovulation remained relatively constant even after 28 days.

2. Ovaries received enough hormone to cause some ovulation even though removed within 22 minutes after the equivalent of five female pituitaries was injected into the body cavity.

3. The number of eggs ovulated by the ovary after it was placed in Ringer's solution was nearly directly proportional to the length of time the ovary was in the body cavity after injection.

4. With low concentrations, from 0.1 to 0.4 pituitaries/ml, ovulation was consistent and increased rapidly with concentration.

5. At concentrations between 0.4 and 0.8 pituitaries/ml, total ovulation was directly proportional to the concentration. This proportionality suggests that, within this concentration range, a direct biological assay of the pituitary hormone can be made by this method.

6. Nearly maximum incidence of ovulation occurred after the injection of a single female pituitary. Increased concentrations of pituitary did not induce significantly greater ovulation; in fact, injections of more than three or four pituitaries seemed to inhibit maximum ovulation.

Retardation of uremic death by desoxycorticosterone acetate. S. RODBARD (introduced by L. N. Katz). *Cardiovascular Dept., Michael Reese Hospital, Chicago.* In the course of previous studies we noted the effect of large amounts of desoxycorticosterone acetate (doca), on uremia in bilaterally nephrectomized animals.

The average duration of life in a large control series after bilateral nephrectomy (one stage) was three days. In six previously uninephrectomized animals the duration of life after total nephrectomy was six days. All of these animals were allowed water ad libitum.

Doca in sesame oil (20 to 50 mgm./day) given

subcutaneously or intramuscularly in fourteen animals led to a longer duration of life after bilateral nephrectomy (one stage), averaging five days. These animals were quiet, showed no thirst or twitching, and vomiting occurred only terminally. The blood NPN rose as in the control dogs. There was a tendency to dehydration indicated by a plasma protein rise. Plasma potassium rose on the average from 16 to about 32 mgm. per cent on the sixth day. The blood pressure was unchanged.

Several factors participate in uremic death in such experiments, viz., trauma of the operation, plasma potassium accumulation, dehydration and hypochloremia from vomiting when animals drink water, accumulation of products normally removed by the kidneys, etc. Doca operates to counteract one or more of these factors.

In two-thirds of the treated animals a progressive fragmentation of subendothelial elastic fibers with fibrinoid degeneration was observed in the pulmonary artery. [*Aided by the A. D. Nast Fund for Cardiovascular Research.*]

Blood pressure studies on dogs. J. M. ROGOFF and E. NOLA NIXON (by invitation). *Laby. of Experimental Endocrinology, School of Medicine, Univ. of Pittsburgh, Penna.* Data were obtained on variations in the blood pressures of 24 normal dogs, at intervals during periods ranging up to 3 years. The "carotid loop" method was found superior to the "arterial puncture" method for comparative observations on blood pressure.

Normal systolic blood pressure ranged between about 100 mm. and 200 mm. Hg. The range for individual dogs varied, over the entire period, within approximately 80 mm. Hg. Variations in blood pressure up to from 30 to 60 mm. Hg may occur in individual animals during a single daily series of successive observations, the average series consisting of 10–12 measurements. Usually, the different readings varied within 5–20 mm. Hg.

In a number of the animals the blood pressure remained at a constant average level for some weeks, then, usually without known cause, became elevated (as much as 60 mm. Hg). In some dogs the blood pressure returned after a while to the original, while in others it remained at the higher level. Thus, in studies on experimental hypertension, elevation of blood pressure should be deemed significant only if it is maintained well above the maximum pressure attained during an adequate control period of observation.

To obtain a minimal significant elevation of blood pressure, by constant intravenous injection of epinephrine, the amounts required are greater than the maximum rate of spontaneous liberation of epinephrine from the adrenals. [*Supported by the G. N. Stewart Memorial Fund.*]

The assay of adrenal cortical extract in adrenalectomized rats exposed to cold. A. ROOS (introduced by E. B. Astwood). *Depts. of Pharmacolggy and Medicine, Harvard Medical School, and the Medical Clinic of the Peter Bent Brigham Hospital, Boston.* A study has been made of certain factors involved in the assay of adrenal cortical extracts by the method described by Selye and Schenker (Proc. Soc. Exper. Biol. and Med. 39: 518, 1938). One day after bilateral adrenalectomy groups of 10–14 young female rats averaging 50 grams in weight were exposed to an environment of 0°C. Colonic temperatures were recorded with a sensitive thermometer at hourly intervals. One half of each group was injected with cortical extract, the other half were uninjected controls. Under these conditions the body temperatures of untreated animals fell to the lethal level of 15–17°C. in 2–5 hours. Wide variability was observed in the individual temperature curves of treated and untreated animals. However, the body temperature proved a more reliable index than did subjective criteria of death. The injected material was considered to exert a protective effect in the dose given if, during the test, a significant difference developed in the average body temperatures of the two groups. Two commercial extracts were used in tests on 300 animals. When extract A was given as a single injection immediately before exposure to cold, a dose of 0.2 cc. was without effect while doses of 0.3 and 0.4 cc. were effective. The minimal effective dose of extract B under identical conditions was 0.05 cc. A second and larger dose of extract given 2–3 hours after the test had begun exerted no protective action while an injection 2–3 hours prior to exposure was effective.

The effect of metrazol convulsions on conditioned reflex training in dogs. VICTOR H. ROSEN (by invitation) and W. HORSLEY GANTT. *Pavlovian Laby., Phipps Clinic, Johns Hopkins Univ.* This study was undertaken for the purpose of examining the alterations produced by metrazol convulsions on the mentally integrated functions of the intact animal measured by the conditioned reflexes.

Four adult dogs were used in whom crude and differential conditioned responses were induced during a controlled training period. In two of the animals the salivary reflexes were used and in the other two motor defense reflexes were studied. After this period a series of ten metrazol convulsions were induced on alternate days with testing of the conditioned reflexes on the interval day. They were also followed for a period of several months following the cessation of the convulsions.

The convulsions appear to disturb the differentiating ability of the dog and to make the inhibitory responses temporarily more active than the excitatory ones. They have a greater effect

on the autonomic salivary reflexes than on the motor defense reactions. It also appears that the overt changes in behavior observed depended upon the original temperament of the animal; there is an apparent improvement in the performance of the "excitatory" animal and a decrease in the efficiency of the performance of the "stable" animal. Furthermore there was a tendency for the differentiating ability to return following the cessation of the convulsions.

The effect of peripheral vasodilation on vasoconstriction: determinations made on the basis of blood pressure of normal subjects. GRACE M. ROTH and CHARLES SHEARD. *Section in Clinical Physiology and Division of Biophysical Research, Mayo Clinic and Foundation, Rochester, Minn.* On twelve normal subjects, observation of blood pressure were made and then in the course of one minute, while the hand of the subject was immersed in water at 4°C., the degree of vasoconstriction was determined by means of the blood pressure. Peripheral vasodilation was then caused to develop in each subject, under resting conditions, through a period of one to two hours. The vasodilation was induced by 1, oral administration of one ounce (30 cc.) of 95 per cent alcohol while the subject was under atmospheric conditions consisting of a temperature of 25.5°C. (78 F.) and a relative humidity of 40 per cent, and 2, at another time, exposure of the subject to an atmospheric temperature of 32.1°C. (89.8°F.), with a relative humidity of 40 per cent. Generalized vasodilation of the peripheral circulation was considered to be present when the temperature of the skin of the toes approximated that of the skin of the trunk. At the height of the peripheral vasodilation the hand again was immersed in water 4°C. and the degree of vasoconstriction was obtained, in order to determine whether generalized peripheral vasodilation could greatly decrease or inhibit the vasoconstriction.

In all individuals tested, irrespective of the basal metabolic rate, and irrespective of the existing generalized vasodilation, the response to the vasoconstricting agent was not significantly altered.

The selective reactions of large and small finger arteries to local cold. LAURENCE W. ROTH (by invitation) and ALRICK B. HERTZMAN. *Dept. of Physiology, St. Louis Univ. School of Medicine.* The following selective reactions occur in the digital artery and the terminal pad arteries on chilling the finger: The digital artery does not participate in the initial constriction which is limited to the terminal pad arteries and which is due to vasomotor reflexes elicited by the cold. The subsequent constriction of the digital artery, which is seldom complete in contrast to that of the terminal arteries, parallels the fall in finger temperature and appears to be due to the direct effect of the latter on this vessel. The reactive dilatation which occurs later during the continued application of cold is limited to the minute vessels of the pad; the digital artery does not participate in this reaction but relaxes when the dilatation in the minute vessels has sufficiently warmed the finger.

These reactions alter the conditions affecting the propagation of the pulse wave in the finger sufficiently to show in predictable changes in the form of the pad pulse. The "crest time" of the latter but not that of the digital artery is shortened during the constriction while a considerable increase over normal in the pad "crest time" and a plateau type of pad pulse may be observed during the earlier stages of the reactive dilatation. These changes are directly related to the filling time of the minute vessels and the degree of constriction of the digital artery. They also indicate how the previously reported changes in the form of the pad pulse are produced in hypertension and arteriosclerosis. [*Aided by the Burgess Battery Co. and the American Medical Association.*]

Human sperm survival in vivo. BORIS B. RUBENSTEIN. *Dept. of Metabolism and Endocrinology, Michael Reese Hospital.* Among the factors affecting fertility of women may be conditions in the female genital tract which influence sperm survival. The literature on sperm survival presents conflicting data, probably because samples were generally taken from the vaginal pool or cervical canal, at haphazard times in the menstrual cycle. The range of survival time reported is from 3 hours to 3 days.

We undertook a systematic investigation of post-coital sperm survival in the vagina, cervical canal, and uterine lumen. Studies were made during the "fertile" periods of 14 menstrual cycles of ten normal young women. Samples of vaginal and cervical secretions obtained before coitus were studied for acidity and microscopic appearance. Similar samples were taken from the vaginal pool and cervical canal promptly *after* coitus and at hourly intervals thereafter. When living sperm were no longer found in these specimens, the cervical canal was swabbed dry and painted with metaphen solution. A canula was introduced into the lumen of the uterus and a specimen withdrawn. This procedure was repeated on the next day. Each post-coital specimen was studied for acidity and for motile sperm.

A summary of our data to date follows. In the vagina, sperm motility was maintained for not more than 3 hours. In the cervical canal, motility was maintained for 4½–7 hours. The uterine secretions rarely contained sperm; whenever sperm were found (once each at 3½, 7, and 25½ hours

post-coitus) they were motile, suggesting that intrauterine conditions favor sperm survival.

This work has been supported by a grant from the Johnson Research Foundation.

The influence of intravenous potassium, calcium and magnesium on the electrocorticogram of the cat. MORTON A. RUBIN (by invitation), HEBBEL E. HOFF, ALEXANDER W. WINKLER and PAUL K. SMITH (by invitation). *The Memorial Foundation of Neuro-Endocrine Research, Worcester State Hospital, Worcester, Mass., Laby. of Physiology, Dept. of Internal Medicine, and the Laby. of Pharmacology, Yale Univ. School of Medicine, New Haven, Conn.* Potassium chloride, calcium chloride and magnesium sulfate in isotonic solution were injected intravenously into cats under nembutal anesthesia. The injections were made at a uniform rate, so that the concentrations of the ions in the serum rose progressively until death. Electrocorticograms from two regions of the brain and electrocardiograms from Lead II were recorded simultaneously throughout each experiment.

Following the administration of potassium or calcium, characteristic electrocardiographic changes appeared. Not until these had progressed to a degree known to be associated with cardiac failure and asphyxia did changes appear in the electrocorticogram. Large, slow waves then gradually replaced the fast waves. Eventually the amplitude and frequency of these large waves declined, although brain potentials could be recorded in all animals for at least 15 minutes after death.

All experiments with magnesium were carried out with artificial respiration. Periods of marked slowing of the waves in the electrocorticogram, alternating with bursts of normal activity, preceded the development of anoxemia due to cardiac failure. In all other respects the course of events resembled that following calcium or potassium. It is concluded that within the range of concentration tolerated by the intact mammalian organism, calcium or potassium are without demonstrable influence on the electrocorticogram, while magnesium may have a depressant effect.

The influence of meat and meat fractions on the fatty liver of the depancreatized and pancreatic duct ligated dog. SAUL H. RUBIN (by invitation) and ELAINE P. RALLI. *Dept. of Medicine, New York Univ., College of Medicine, New York City.* Liver lipids and their fractions were determined in depancreatized and pancreatic duct ligated dogs. None of the animals received any lipotropic supplement such as choline or lipocaic or raw pancreas or pancreatic juice. The basic diet consisted of cracker meal, milk powder, salts, yeast, corn oil and cod liver oil. In addition to the basic diet 7 dogs received 100 to 150 grams of raw beef daily; 15 received a dried meat powder preparation from which the extractives had been removed; 7 received the dried meat powder plus a concentrated meat juice; 3 received the dried meat powder plus cystine (0.1 gm. to 2 and 0.2 gm. to 1 dog daily); and 3 received dried meat powder plus inositol (2 gms. to 2 and 1 gm. to 1 dog) daily.

The dogs on raw beef were studied for 3 to 25 weeks; 3 were depancreatized, 4 were duct ligated. The liver lipids were greatly increased in 6 dogs. The dog sacrificed after 3 weeks had 8.9 gms. per cent liver lipids; the average liver lipids in the other 6 were 24.5 gms. per cent.

Twelve of the dogs fed meat powder were depancreatized. The period of observation varied from 6 to 43 weeks. The average total liver lipid was 7.5 gms. per cent and varied from 3.8 to 13 gms. per cent. Only 4 dogs had liver lipids above 10 gms. per cent.

The dogs on meat powder plus meat juice were followed from 5 to 24 weeks; 6 were depancreatized. The average total liver lipid was 10 gms. per cent with a range from 4 to 22 gms. per cent.

The dogs given cystine were observed for 13 to 19 weeks. The total liver lipids were 14, 28 and 6 gms. per cent. The latter was in a dog on 0.1 gm. of cystine.

The dogs given inositol were followed from 9 to 22 weeks. The total liver lipids were 6, 20, and 27 gms. per cent. The low total lipid was in the dog on 1 gm. of inositol.

Hyperphagia and adiposity in relation to disturbances of taste. T. C. RUCH, H. D. PATTON (by invitation) and J. R. BROBECK (by invitation). *Laby. of Physiology, Yale Univ. School of Medicine, New Haven, Conn.* Determinations of the taste threshold for bitter solutions by the "preference method" have been made in a series of monkeys before and after bilateral Horsley-Clarke lesions in the medial posteroventral region of the thalamus and the adjacent mesencephalic tegmentum. Eating behavior and weight changes have also been followed. Nearly every combination of taste disturbance and hyperphagia-adiposity has been found. Of three animals showing hyperphagia the disturbance of taste varied from slight (doubling of the threshold) to a marked interference with taste; all three have exhibited pronounced motor abnormality especially of jaw movements and reflexes. The monkey showing greatest taste deficit exhibited no adiposity nor disturbance in the motor sphere. In two experiments neither function was affected. Taste disturbances and the hyperphagia-adiposity appear to be separately localized and not casually related. [*Aided by a grant from the Fluid Research Fund, Yale University School of Medicine.*]

Carbohydrate metabolism in the eviscerated

rat, II. JANE A. RUSSELL and JAY TEPPERMAN (by invitation). *Dept. of Physiological Chemistry, Yale Univ. School of Medicine, New Haven, Conn.* Studies on the part played by the anterior pituitary in carbohydrate metabolism after evisceration have been extended as follows:

The amount of glucose required to maintain constant blood sugar levels in eviscerated normal rats has previously been found to be about 13.5 mgm. per 100 gm. (initial weight) per hour. In normal eviscerated rats treated with saline anterior pituitary extract, the glucose requirement is reduced to about two-thirds of this amount. Experiments have shown that the liver which remains in situ after the ligation and severance of all its afferent blood vessels does not contribute carbohydrate to the rest of the body. Either less carbohydrate is oxidized peripheraly, or less is deposited as glycogen, in the anterior pituitary treated eviscerated rat than in the untreated animal.

The R. Q.'s of eviscerated adrenal-demedullated rats given glucose subcutaneously have been determined. In these cases, enough glucose was administered so that the blood sugar levels prevailing at all times were above normal. The R. Q.'s of normal rats lay near 0.85. Those of hypophysectomized rats identically treated were generally above 0.90, and the mean R. Q. of the latter animals greatly exceeded that of the normal. Changes in blood lactic acid were small or absent in both series of experiments. The difference between the R. Q.'s obtained in intact glucose-fed (or fasted) hypophysectomized rats and those in normal rats is thus evident also in eviscerated animals. [*Aided by grants to Dr. C. N. H. Long from the Committee on Research in Endocrinology, National Research Council.*]

The effect of insulin, thyroxin and their combination on the energy metabolism of the rabbit. R. RYER (introduced by J. R. Murlin). *Dept. of Vital Economics, Univ. of Rochester, Rochester, N. Y.* The standard doses of thyroxin and insulin have been one half milligram per killogram per week and one half unit per killogram twice a day, respectively.

This dose of thyroxin increases the energy metabolism to a plus thirty per cent mean value for the week, with a peak of plus fifty to sixty per cent at three to four days which gradually disappears with a return to normal in seven to nine days. There is a corresponding fluctuation in protein metabolism.

This dose of insulin causes only a qualitative shift in metabolism with no regular increase in energy output. There is a marked protein sparing effect concurrent with the depression of the blood sugar. With the succeeding rise in blood sugar, there is a corresponding rise in protein metabolism.

The combination of these hormones brings about a ten per cent enhancement of the effect of thyroxin. There is, however, the usual insulin protein sparing effect which becomes more marked as the percentage of protein being metabolized increases in response to the administered thyroxins.

Mechanical changes of muscle during the latent period of isometric contraction. ALEXANDER SANDOW (introduced by Robert Chambers). *Washington Square College of Arts and Sciences.* Using a piezo-electric cathode-ray oscillographic technique, the existence of a latency relaxation (L.R.) ("Rauhsche Nase," Rauh, 1922; Roos, 1932; Schaefer and Göpfert, 1937) has been confirmed. The method permits the determination in a single contraction of a number proportional to the maximum velocity of this relaxation (M.V.) and the times from the instant of stimulation to 1, the beginning of L.R. (L_R); 2, the attainment of M.V. (T.M.V.); 3, the instant when the relaxation changes over to positive tension development (L_T). Characteristic time values for the frog sartorius previously soaked in pH 7.0 buffered Ringer's solution, at 20°C., stimulated at *in situ* length with maximal shocks, are: L_R = 1.65, T.M.V. = 2.92, and L_T = 3.30 ms. ($\sigma = \pm$ 0.03 ms.). These time values and M.V. each change in a regular fashion among the contractions of an activity series comprised of maximum twitches at 4 sec. intervals for 3 mins. and during the subsequent recovery period. During the activity series M.V. continuously decreases, T.M.V. and L_T first increase and then decrease, and L_R seems to remain constant. During the recovery period (30–45 min.) all quantities tend to return to their initial values. These latency alterations may be correlated with the changing internal chemical environment of the muscle resulting from its own activity; and the nature of the correlation suggests that the hydrolysis of adenylpyrophosphoric acid is the immediate source of energy for contraction.

The action of posterior pituitary extract on the dog heart. MARGARET E. M. SAWYER and G. H. ETTINGER (by invitation). *Dept. of Physiology, Queen's Univ., Kingston, Ontario.* The slowing of the unanaesthetized dog's heart, produced by intravenous injection of posterior pituitary extract, is entirely due to stimulation of the vagal and sympathetic cardio-inhibitory fibres. This is shown by the following experiments: *a.* In the normal dog continuous infusion of a dilute solution of posterior pituitary extract produces maximal inhibition, (slowing of the heart rate to one half the resting rate), with a rise in blood pressure of only 10–15 mm. Hg. The slowing is therefore

unlikely due to a carotid-aortic-sinus reflex. It is abolished by atropine. *b*. After bilateral thoracic sympathectomy posterior pituitary extract produces maximal inhibition. This is abolished by atropine. *c*. After double vagotomy posterior pituitary extract produces only a moderate inhibition with a slight rise in blood pressure. This inhibition is not abolished by atropine. Apparently the sympathetic inhibitory fibres may be excited by the extract but not blocked by atropine. *d*. After total denervation of the heart posterior pituitary extract produces no inhibition. *e*. Characteristic changes are produced by posterior pituitary extract in the electrocardiogram of normal dogs. After total denervation no change takes place.

It has not been determined whether the slowing is due to a central or reflex action. If it is reflex, afferent fibres must travel through both sympathetic and vagal pathways. Constriction of the coronary arteries is a factor only if this stimulates a reflex nervous mechanism. *Aided by a grant from the National Research Council, Ottawa.*

The production of traumatic shock in dogs after total sympathectomy and after spinal cord transection. A. E. SCHECTER (by invitation), M. L. CULLEN (by invitation) and N. E. FREEMAN. *Harrison Dept. of Surgical Research, Schools of Medicine, Univ. of Pennsylvania, Philadelphia.* The sympathetic nerves were removed in one group of dogs, and the spinal cord was transected in another group of dogs. When recovery from the operations had occurred the animals were studied in the following manner: The muscles of one hind leg were traumatized by the technic of Best and Solandt and the bones of the extremity were fractured. Ether anesthesia was used in the sympathectomized dogs. Blood loss into the area of injury was restricted by binding the extremity. Blood pressure, peripheral blood flow through an uninjured paw, hemoglobin, hematocrit, arterial and venous blood gas concentrations, cardiac output and blood volume determinations were made before and within three hours after trauma. The loss of blood into the area of injury was measured by a dissection technic, and the visceral organs were examined after death.

Hemoconcentration and a reduction of blood volume occurred despite the absence of excessive blood loss into the injured extremities and in the presence of a well maintained circulation as measured by peripheral blood flow, cardiac output and venous oxygen saturation of blood obtained from the right heart. Post-mortem examinations revealed pathological changes characteristic of shock. These findings suggest the presence of some factor capable of causing a reduction of blood volume not primarily due to excessive local fluid loss or to a reduced circulation.

The effect of urogastrone on gastric secretion in enterectomized dogs. M. J. SCHIFFRIN (Porter Fellow in Physiology, 1941–'42) (by invitation) and JOHN S. GRAY. *Dept. of Physiology and Pharmacology, Northwestern Univ. Medical School, Chicago.* The entire small intestine from the pylorus to the ileocecal sphincter was removed under aseptic conditions in 6 dogs. The body of the pancreas was removed, the common duct ligated and external drainage of the stomach was established. The dogs were maintained postoperatively by the subcutaneous administration of a modified Locke's solution every 6 hours.

Beginning two days after the operation, gastric secretion was stimulated by the subcutaneous injection of 0.15 mgm. histamine dihydrochloride at 10-minute intervals and was collected in 20-minute periods. When the rate of secretion remained constant for 3 collection periods, 1.0 mgm. urogastrone was injected intravenously. In all the 12 experiments, the rate of secretion and the output of free HCl were reduced. The maximum effect was observed during the second collection period following the administration of urogastrone. During the first hour after the injection, the average inhibition of the volume of secretion was 50 per cent, and that of the free HCl output, 59 per cent. In 8 similar experiments performed on 8 dogs with total gastric pouches in whom the small intestine had not been removed, the average inhibition of the volume of secretion was 35.4 per cent, and that of the free HCl output, 39.9 per cent.

These experiments indicate that the inhibitory effect of urogastrone on gastric secretion is not dependent upon the small intestine.

Inhibition of the sulfonamide effect on pneumococcus by p-aminobenzoic acid in the absence of oxygen. C. SCHLAYER (by invitation), W. KEMPNER and P. SUMMERS (by invitation). *Dept. of Medicine, Duke Univ., Durham, N. C.* One-tenth cubic centimeter of a 2 to 3 hour old pneumococcus type I culture was transferred to 20 cc. of beef-infusion broth pH 7.4, containing per 100 cc. 20 cc. human serum, 0.8 g Difco-Bacto-peptone, 0.4 g glucose and, for the anaerobic determinations, in addition 0.21 g $NaHCO_3$. Oxygen consumption

	INHIBITION OF PNEUMOCOCCUS GROWTH AFTER A GROWTH PERIOD OF 280 MINUTES		INHIBITION OF THE EFFECT OF SULFATHIAZOL ($2 \cdot 10^{-4}$M) BY p-AMINOBENZOIC ACID ($0.15 \cdot 10^{-4}$M)
	$2 \cdot 10^{-4}$M sulfathiazol	$2 \cdot 10^{-4}$M sulfathiazol $0.15 \cdot 10^{-4}$M p-aminobenzoic acid	
	per cent	*per cent*	*per cent*
Air	71	33	53.5
Nitrogen	88	47	46.5

in air and CO_2 formation in 5 per cent CO_2/N_2 were measured by the Warburg technique at 38°C using 2 cc. samples of the culture, with and without the addition of various concentrations of p-aminobenzoic acid and of various sulfonamides. The cultures reached maximal growth after 240 to 300 minutes.

The anti-sulfonamide effect of p-aminobenzoic acid, found by Woods and other authors with bacteria growing under aerobic conditions, is evident in pneumococcus also in the complete absence of oxygen, and is of the same magnitude in nitrogen as in air.

Chemical composition of human prostatic and vesicular fluids and of semen. WILLIAM W. SCOTT (by invitation), CHARLES HUGGINS and J. HENRY HEINEN (by invitation). *Dept. of Surgery, Univ. of Chicago.* Quantitative determinations were made of pH and of water, electrolyte and protein concentrations of the semen and of the fluids which are its principal constituents.

Most of the proteins are proteoses which are soluble in hot water and dialyze freely through cellophane. The total protein concentration is less than that of blood serum. The proteoses arise in both the prostate and seminal vesicle. The protein concentration is reduced in hypogonadism and by frequent ejaculation. A low protein content of semen is accompanied by an increase of chloride.

The seminal vesicle secretion is a heavy fluid, rich in protein and NPN constituents. The prostatic secretion is a light fluid with much less protein; the concentration of K is high, while that of chloride and bicarbonate is low.

The rôle of the liver in the metabolism of a-Estradiol. ALBERT SEGALOFF (by invitation) and WARREN O. NELSON. *Wayne Univ., College of Medicine, Detroit.* In an evaluation of the activity of the liver in the metabolism of estrogens, intrasplenic injections were made of a-estradiol and its esters. Controls were injected subcutaneously. In one group of rats injections were made intrasplenically with spleens *in situ* so that venous drainage was through the portal system. In a second group injections were made into spleens which had been transplanted subcutaneously so that they drained into the systemic circulation.

A comparison of the potency of estrogens injected into the spleen *in situ* revealed a decreasing order of effectiveness as follows: a-estradiol-benzoate-butyrate, a-estradiol dipropionate, a-estradiol benzoate and free a-estradiol. The approximate ratios of the amounts of these substances necessary to produce estrus in 50% of the animals were: 1: 2: 10: 20.

However, when the ratio of efficiency in the transplanted spleen to that in the intact spleen was compared, it was found that a-estradiol benzoate was about equal in potency to the free hormone. The dipropionate and the benzoate-butyrate continued to show more activity than other estrogens. On the basis of ratios the dipropionate is 5 times and the benzoate-butyrate 50 times as effective as the free hormone when drainage is through the portal system.

These results indicate that the liver attacks a-estradiol on its hydroxyl groups and that esters of a-estradiol are at least partially absorbed from the spleen as such since esterification increases the effectiveness of estrogens which have been injected into the spleen *in situ*.

Further studies concerning the anesthetic action of steroid compounds. HANS SELYE. *Dept. of Anatomy, McGill Univ., Montreal, Canada.* A systematic study of 80 steroid compounds assayed on the partially hepatectomized rat according to the technic described by Selye (J. Pharmacol. and Exper. Therap. **71**: 236, 1941) revealed that anesthetic action is demonstrable in the case of every hormonally active steroid irrespective of the nature of its hormonal effect.—The highest anesthetic action is exhibited by steroids oxygenated only at the two extreme poles of the molecule, that is at C_3 and C_{17} in the case of androstane and etiocholane derivatives and at C_3 and C_{20} in the case of pregnane and *allo*-pregnane derivatives.—One double bond does not appear to interfere seriously with the anesthetic effect if it is situated in ring A or B, but two or more double bonds in these two rings, or one double bond in ring D are detrimental.—Esterification decreases the anesthetic effect only if it delays absorption, while the reverse is true in the case of particularly soluble readily absorbable esters.—The steric position of a hydroxyl group in position C_3 appears to be without importance for the anesthetic effect at least in the androstane and etiocholane series.—A five-membered ring D is not indispensable for the anesthetic effect as shown by two active chryso-pregnene derivatives.

Rod and cone dark adaptation in total color blindness and in macular degeneration. CHARLES SHEARD and H. L. BAIR (by invitation). *Division of Biophysical Research and Section on Ophthalmology, Mayo Foundation and Mayo Clinic, Rochester, Minn.* In a case of congenital total color blindness, dark adaptation of the macula (cones) followed the same course of threshold levels as is obtained normally in peripheral (chiefly rods) regions. Peripheral adaptation was somewhat better (about 0.5 log unit) than is found in the average normal subject.

In a case of bilateral macular degeneration, the same type of curves and comparable threshold levels were obtained for dark adaptation in peripheral areas and at the macula, the peripheral adaptation being normal.

The conclusion is drawn from these findings and the data to be presented that cone adaptation primarily was affected, with "rod" function in areas (macular) which normally are populated entirely by cones, together with an apparent enhanced functioning of the rods in peripheral regions.

Operative mechanism of some errors in the thermostromuhr method. R. E. SHIPLEY (by invitation), D. E. GREGG and J. T. WEARN. *Dept. of Medicine, Western Reserve Medical School, Cleveland, O.* In using the direct current thermostromuhr (Baldes and Herrick type) several factors other than rate of flow may influence the empirical flow readings (namely, changes of the thermostromuhr environment, presence of zero flow, and back flow in the flow pattern.

The operative mechanism of the thermostromuhr was therefore examined from the standpoint of qualitative changes in the heat gradients about the thermocouples. Fundamentally, both thermocouples, heated primarily by direct conduction through the block from the heater are *cooled* by the fluid passing through the unit. The extent to which each is thereby heated and cooled in relation to flow determines the coincident galvanometer deflection/flow relation, or so-called calibration.

External environmental changes alter the thermocouple temperatures through variation in rate of heat loss to the environment.

Internal environmental change caused by introduction of zero flow or back flow in the flow pattern of the fluid metered, precludes the use of a conventional calibration because of the accompanying redistribution of heat about the thermocouples.

A calibration of a thermostromuhr is applicable to its operation only under environmental conditions identical to those under which it is calibrated.

Studies of turbulence *in vitro* indicate that the extent to which turbulence may naturally exist in small vessels is insufficient to produce effects worth consideration in the face of the more demonstrable and detectable influences above which impair the instrument's accuracy. [*Supported by a grant from the Commonwealth Fund.*]

The effect of menarche on basal physiological functions in girls. NATHAN W. SHOCK (introduced by Harold F. Blum). *Inst. of Child Welfare, Univ. of California, and National Inst. of Health, U. S. Public Health Service, Bethesda, Md.* Determinations of pulse rate, blood pressure, respiratory volume and basal heat production were made on each of 50 normal girls at intervals of six months between the ages of 11.5 and 17.5 years. All determinations were made under basal conditions on two successive mornings at each six month testing. The Tissot open circuit method was used for estimating basal oxygen consumption during three 8-minute periods on each of the two mornings. Accurate menstrual histories were kept for each subject.

Analysis of curves relating the measured functions with age for each girl, shows three marked changes following within six months the inception of menstruation: 1, a leveling off of a pre-menarcheal rise in systolic blood pressure; 2, a decrease in pulse rate; 3, a precipitous fall in basal heat production per unit of surface area. None of these phenomena are observable in average growth curves based on chronological age because of the different age at which the onset of menstruation occurs among normal girls.

The differential effects of respiration on the left and right ventricles. ROBERT H. SHULER (by invitation), CHARLES ENSOR (by invitation), R. E. GUNNING (by invitation), W. G. Moss (by invitation), and VICTOR JOHNSON. *Dept. of Physiology, Univ. of Chicago.* Previous findings on the effects of respiration on cardiac filling and output have been contradictory, probably because the oncometer method necessitates measurements of the combined stroke of both ventricles. In an attempt to differentiate the behavior of the two ventricles, large dogs were anesthetized with sodium barbital, a portion of the ventral chest wall removed, the heart exposed, and paper markers affixed to the heart so as to outline each ventricle. A window was sealed into the ventral chest opening, normal respiration was reinstated, and motion pictures were taken of the heart. Successive single frames were projected, the area of each ventricle measured in each frame, and these areas graphed together with simultaneous intrathoracic and carotid pressures. The right ventricle showed increased diastolic size and stroke volume during inspiration, while the same measurements on the left ventricle decreased, though to a lesser extent. Although more blood is aspirated into and ejected by the right ventricle in inspiration, this blood is withheld from the left ventricle in the expanded lung vessels, so that at this time the left ventricular diastolic and stroke volumes (plus also the arterial blood pressure) decrease.

Each ventricle responded independently according to the Starling principle, regardless of the diastolic size of the other ventricle.

Effect of posture on the duration of Pentothal anesthesia. HERBERT SILVETTE. *Dept. of Pharmacology, Univ. of Virginia, Charlottesville.* (Read by title.) While determining the effect on diuresis of Pentothal-sodium, it was observed that the position in which anesthetized rats were placed had a marked influence on the duration of anesthesia.

The time of reappearance of reflex righting

movements of *a*, forelimbs; *b*, hind-limbs, and *c*, of such movements so co-ordinated as to enable the animals to regain the normal position were determined in rats anesthetized with Pentothal (6 mgm. per 100 grams, intraperitoneally) and placed in either the prone or supine position. In 27 rats in the *prone* position, the time elapsing before the above-described righting movements reappeared were, respectively, 205 ± 8.9, 217 ± 8.7 and 321 ± 8.5 minutes (Averages ± Probable $\text{Error}_{\text{Average}}$). For 17 animals in the *supine* position, the respective times were 295 ± 10.1, 322 ± 10.3 and 401 ± 9.9 minutes. Thus, in the supine position the fore-limb and hind-limb movements and righting time were delayed by 43, 49 and 25 per cent, respectively. These differences are highly significant statistically.

Other rats, deprived of both food and water for 24 hours, were also tested as to their responses while in the prone and supine position after Pentothal injection. Supine animals showed considerably delayed righting movements in both forelimbs and hind-limbs. A further group injected with sodium chloride solution and then anesthetized with Pentothal showed similar prolongations of righting time when supine as compared with prone. Both dehydration and hydration led to definite increases in righting time for fore-limbs and hind-limbs compared with control animals.

The influence of vitamin B (complex) surplus on the capacity for muscular and mental work. ERNEST SIMONSON, ALBERT BAER (by invitation) and NORBERT ENZER (by invitation). *Mount Sinai Hospital and the Lakeside Labs., Milwaukee, Wis.* Is the amount of Vitamin B in the average diet of normal people sufficient to guarantee the maximum capacity for physical and mental work? The effect of a large surplus of Vitamin B complex (6 mgm. Thiamin, 80 mgm. Nicotinamide, 8 mg. Riboflavin, 0.3 mgm. Pyridoxine, 80–120 units filtrate factor a day), given for 6 to 10 weeks after a preliminary period of three weeks, was studied in 12 healthy subjects as compared with a control group of 11 subjects. No effect of Vitamin B intake was seen in five different types of muscular work: dynamic work (endurance and recovery), static work (endurance and recovery), maximum frequency of finger movements, absolute muscle force, fatigue of maximum muscular effort. The increase and recovery of pulse rate in dynamic and static work was not changed. However, there was a pronounced increase of the fusion frequency of flicker (which is intimately related to fatigue of the central nervous system) in 8 of 12 subjects, beginning after three weeks intake and reaching the maximum after 5 weeks intake. The fusion frequency drops to the initial values during four to six weeks after cessation of intake. The placebos given to the control group have no effect. All 12 experimental subjects and only one of the control group felt improvement of subjective working capacity. Conclusion: A favorable influence on the capacity for all types of work with prevailing fatigue of the central nervous system might be expected in the majority of normal people from the intake of considerable Vitamin B complex surplus.

Concerning the toxic factor in traumatic shock. HOWARD SLOAN and RALPH K. KLAUS (introduced by L. B. Nice) *Chicago Medical School.* (Read by title.) Using etherized dogs and recording the blood pressure from a carotid artery, the middle sacral artery was cannulated; a hemostat was placed across the junction of the middle sacral and right common iliac arteries so that there was no blood flow into either, but Ringer's solution perfused through a cannula in the middle sacral artery would enter the right common iliac, and perfuse the traumatized limb.

The right hind leg was traumatized by striking it fifty times with a heavy iron pipe, breaking the femur. The leg was then perfused with 250 cc. of Ringer's solution letting this solution go into the general circulation. No fall in blood pressure occurred during transfusion. The hemostat on the right common iliac artery was then removed letting the blood flow through the traumatized area. The blood pressure fell gradually to shock level and the animals died.

In a second series of experiments the right common iliac vein, internal iliac, hypogastric, middle sacral and all superficial abdominal veins were ligated.

The common iliac artery was opened after traumatizing the leg letting the blood enter. The blood pressure gradually fell to shock level and the animals succumbed, with no return of toxic blood through the traumatized area to the general circulation.

The hematocrit showed hemoconcentration.

There was no indication of nervous shock during traumatization.

By weight the isolated traumatized leg was considerably heavier than the isolated uninjured left hind leg due to the accumulated fluid which apparently caused the fall in blood pressure and shock.

Monocular and binocular fusion in movement and depth perception after section of the commissures of the cerebral cortex. KARL U. SMITH (introduced by Wilbur K. Smith). *Univ. of Rochester.* Dr. Charles S. Bridgman coöperated in this study.

Experiments have been made on the rôle of the cortical commissures in perceptions produced by stimulation of the heteronymous retinal fields. Patients of Dr. William P. Van Wagenen, The University of Rochester School of Medicine and Dentistry, were observed. These patients, all

epileptics, had undergone remedial neurosurgery involving partial or complete section of the corpus callosum. In some few cases other cortical commissures were sectioned as well.

Five tests (detecting apparent-movement monocularly, a special test of stereoscopic depth perception, a vertical-peg test of depth perception, determination of the prism power for binocular fusional breakdown, and determination of the level of binocular fusional recovery from diplopia produced by prisms) were employed, all of which depended upon fusion of disparate activity set up in the heteronymous halves of the retinae.

Separation of the two cortical visual areas by cutting their interconnecting fibers in the splenium of the corpus callosum presumably should disturb performance in all of the test situations. This was true only in the case of the last test, in which case there was a slight but consistent reduction in performance.

Arguments based on the results will attempt to show that the neural fusion associated with binocular and monocular perception has its locus in subcortical mechanisms of vision.

Evidence of a representation of tactile sensibility in the cerebellum of the cat. RAY S. SNIDER and AVERILL STOWELL (introduced by Philip Bard). *Dept. of Physiology, School of Medicine, The Johns Hopkins Univ., Baltimore, Md.* In cats under nembutal anesthesia a discrete tactile stimulus produces a well localized potential wave in certain regions of the cerebellar cortex. Maximal responses are found only on the side ipsilateral to the stimulus and are confined to the anterior lobe and the paramedian lobule. Smaller potentials have been recorded from these same parts on the contralateral side and from the medial portions of crus I and crus II of the ipsilateral hemisphere. Different parts of the body appear to have distinct representations in the parts of the cerebellum mentioned and a map of this arrangement will be presented. One of the most distinct representations is that of the toes of the forefoot. Tactile stimulation of this part of the periphery gives maximal responses in 1, the most lateral part of the fourth folium of the paramedian lobule, and 2, that portion of the culmen which is made up of the fourth folium anterior to the primary fissure. The loci and latencies of the responses are not altered by decerebration at a high mesencephalic level. The latencies range from 10 to 15 milliseconds. These localized evoked cerebellar responses are primarily surface positive, but occasionally surface negative and diphasic waves are observed.

The potential changes were recorded by means of a 3 stage resistance-capacity coupled amplifier and a cathode ray oscillograph. A moist cotton thread drawn through steel tubing served as pick-up electrode and the indifferent electrode was placed in contact with the scalp or skull.

Simple electric integration of action potentials as a direct index of muscular activity. J. M. SNODGRASS (introduced by Birdsey Renshaw). *Oscillograph Laby., Dept. of Psychology, Oberlin College, Oberlin, O.* Simple electrical circuits are used to rectify and integrate muscle action potentials. The time constants may be readily varied to suit the problem at hand. The integrated tracings facilitate the reading of action current pulses associated with muscle contractions which may be superimposed upon a background of general muscular activity. Integrated tracings may be directly equated with the degree of muscular contraction. Applications and limitations of the method will be discussed.

The oxygenation and carbon dioxide content of fetal blood during the interruption of fetal respiration coincident with the breathing of low oxygen mixtures by the maternal animal. FRANKLIN F. SNYDER. *Depts. of Obstetrics and Gynecology and of Pharmacology, Univ. of Chicago, Ill.* The experimental production of apnea for intervals as desired in fetuses showing rhythmical intrauterine respiratory movements revealed certain differences in the regulation of fetal respiration in contrast to that of the maternal animal, especially with regard to the effect of low oxygen. In the present experiments in rabbits prepared for direct observation of the fetuses after laparotomy in a saline bath, blood samples were drawn from the umbilical vein during fetal apnea which was experimentally induced by the administration of a low oxygen mixture (4 per cent oxygen in nitrogen) to the mother for several minutes. In each sample of 0.25 cc. the oxygen content, carbon dioxide content and hemoglobin concentration were determined at once. In addition blood samples from the umbilical veins were taken during the occurrence of rhythmical fetal respiratory movements both preceding and after the administration of low oxygen to the mother. About fifteen minutes elapsed between venapunctures. Observations were based upon examination of 23 fetuses obtained from nine litters.

Results of blood analyses showed that there was a striking decrease in the oxygenation of fetal blood during the induced apnea, the oxygen saturation often falling to one third of that observed before and after induction of apnea; during apnea the oxygen content averaged about 4 volumes per cent. No marked changes in carbon dioxide content were noted during apnea. Thus, direct sampling of fetal blood revealed anoxemia coincident with depression of fetal respiratory movements while at the same time the maternal animal showed respiratory stimulation.

Changes of conductance and of cellular per-

meability induced by electrical stimulation of the brain. E. SPIEGEL, M. SPIEGEL-ADOLF (by invitation) and G. HENNY (by invitation). *Depts. of Experimental Neurology, Colloid Chemistry, D. J. McCarthy Foundation, and the Dept. of Physics, Temple Univ. School of Medicine, Philadelphia, Pa.* The electrical conductance of the brain following faradic stimulation was studied on cats in vivo and on surviving frog's brains. In measurements with the circulation intact the associated changes in the blood supply of the brain are a complicating factor. This could be eliminated by measurements on isolated surviving frog's brains. In these an increase of the conductance could be demonstrated following the stimulation with faradic current. Further experiments showed that this phenomenon is of complex nature; it is caused partly by a rise of temperature induced by the stimulating current, partly by a decrease of the density of the cellular surface films and the subsequent leakage of intracellular ions into the intercellular spaces. The appearance of such changes in the brain cells following stimulation could be demonstrated in a further series of experiments on isolated frog's brains, which were stimulated while bathed in an isotonic glucose solution. The conductivity of this bathing fluid was distinctly increased following the stimulation indicating a leakage of ions from the brain into the surrounding fluid as a consequence of the stimulation.

Comparison of the effects of iodoacetate and iodoacetamide on the respiration of caffeinized frog muscle. J. N. STANNARD. *Dept. of Pharmacology, Emory Univ. Medical School, Emory University, Ga.* In an earlier publication (Am. J. Physiol. **122**, 1938) a study was made of the action of iodoacetate and iodoacetamide on the respiration and glycolysis of resting muscle. Since more recent work by the author has indicated differences between the enzyme systems utilized by resting as compared with active muscle (electrical or chemical stimulation), a study of the action of these compounds on the respiration of active muscle (stimulated by 0.04% caffeine) was considered of interest.

The oxygen consumption of caffeinized muscle was much more sensitive to both iodo compounds than was that of resting muscle. In addition the times or concentrations necessary to produce a given effect were of the same order as those required for inhibition of the resting glycolysis. At concentrations above 0.001M the two compounds were about equally effective inhibitors of the activity respiration. Below 0.001M the iodoacetate became considerably more rapid in its action than iodoacetamide. This is the same order of effectiveness as that found for inhibition of glycolysis and stands in contrast with the situation encountered when the O_2 consumption of *resting* muscle was measured. It seems possible, therefore, that 1, inhibition of the activity respiration depends only upon inhibition of glycolysis; 2, different enzyme entities are involved in the action of these compounds on the resting and activity respirations, and/or 3, the presence of caffeine alters the sensitivity of the respiration to the iodoacetyl group, directly or by changes in differential permeability. Experiments are now in progress to test these possibilities further.

Observations on the concentration and absorption of minerals by the rectal mucosa. F. R. STEGGERDA. *Dept. of Physiology, Univ. of Illinois, Urbana.* In view of the markedly richer blood supply to the rectum than to the colon mucosa just proximal to the rectum, it was assumed that there might also be a difference in the concentration of various minerals in the faecal material that had been in contact with these two regions previous to defecation.

To test this hypothysis, a large number of human stools were collected and divided into colon and rectal portions and then analyzed for moisture, ash, calcium, phosphorus, iron, and chlorine. The results show that there is 7% less water in the rectal end than in the colon end, while the percentage ash is smaller in the rectal end. The percentage concentration of calcium, phosphorus, and iron is greater in the rectal end, whereas the reverse is true for the chlorine content.

These percentage differences in ash and mineral concentration between the two ends of the stool, though small, are highly significant and are evidence that chlorine is absorbed along with water in the rectum while calcium, phosphorus, and iron are increased in the rectum because of the absorption of water and not active secretion via the rectal mucosa.

No percentage difference is produced in calcium, phosphorus, and iron between the two ends of the stool following high or low intake of such elements. Experiments on dogs, where the difference in moisture content between the two ends of the stool was less than 2 per cent, showed no difference in mineral concentration as occurred in the human stool.

Acclimatization to simulated altitudes in relation to gastric emptying. J. CLIFFORD STICKNEY (by invitation) and EDWARD J. VAN LIERE. *Dept. of Physiology, School of Medicine, West Virginia Univ., Morgantown.* It is known that anoxia delays gastric emptying. The authors studied the effect of discontinuous exposure to anoxia upon this delay with the hope that another criterion of acclimatization would be found.

Normal gastric emptying times were determined fluoroscopically on five dogs according to procedures previously used in this laboratory. The dogs were then exposed daily (except Sundays) to

simulated altitudes by means of a low pressure chamber. The length of the daily average exposure was about 8 hours.

Four of the dogs showed an average delay in gastric emptying of approximately 35 per cent at 12,000 ft., but with continued daily exposure all dogs eventually regained their normal values. Three of the dogs were then exposed to an altitude of 16,000 ft. (causing a delay in gastric emptying of about 40 per cent), and were subjected to this altitude daily until normal values were attained. Finally all the dogs were exposed to an altitude of 18,000 ft. (with a delay of about 60 per cent). But one dog of the five showed definite attainment of normal values at this altitude.

Considerable individual variation (3 to 8 weeks) was found in the development of acclimatization as measured by gastric emptying time. Hemoglobin and erythrocyte values, which were determined periodically, rose gradually during the work. The experiments showed that during acclimatization gastric emptying time gradually returns to the normal, either partially or completely, and that it may be regarded as another criterion of acclimatization.

Evidence of a representation of auditory sensibility in the cerebellum of the cat. AVERILL STOWELL and RAY S. SNIDER (introduced by Philip Bard). *Dept. of Physiology, School of Medicine, Johns Hopkins Univ., Baltimore, Md.* It has been found that in cats under nembutal anesthesia click stimuli give rise to discrete electrical responses in certain regions of the cerebellar cortex. The responses are primarily surface positive and may or may not be followed immediately by a smaller negative component. Maximal potentials are found in the most medial portion of the ipsilateral half of the lobulus simplex. On moving the pick-up electrode laterally over this lobulus the responses become smaller and smaller and disappear entirely at the margin of crus I. Small responses are found contralaterally in the lobulus simplex and bilaterally in the anterior portion of the declive and the most caudal portion of the culmen. These small responses are similar in type to those found in the ipsilateral lobulus simplex and all the potentials resemble the waves set up in the inferior colliculus by auditory stimuli. Responses of somewhat different character have been recorded from the most rostral portion of the anterior lobe. All the responses are entirely masked by continuous sound and are abolished by removal of the inferior colliculi. The latencies of the waves vary from 11 to 14 msec.

The methods of recording the potentials were the same as those described in the accompanying abstract by Snider and Stowell.

A lateral localization in cat's visual cortex. S. A. TALBOT. *Laby. of Physiological Optics, Wilmer Ophthalmological Inst., Johns Hopkins Univ. and Hospital, Baltimore, Md.* The cat's visual vertical meridian follows a line diagonally along the lateral gyrus in the lower field, and into the bottom of the postlateral sulcus in the upper field. The whole contralateral visual field is represented (binocularly) medial to this line. A *second* functional localization appears laterad to this line, oppositely disposed, and confined anteriorly to the lateral gyrus, posteriorly to the suprasylvian. The second representation, equally accurate, seems to cover a narrow visual band, especially occipitally. Here the exposed cortical surface affords double access to points in a small area of the upper visual field. For other field points, one or both corresponding cortical loci are buried.

General stimulation (optic-nerve shock or retinal flash) produces similar responses from these two areas. Localized photic stimuli also give responses virtually identical, in: 1, latency of primary wave; 2, separability of the diffuse-light wave by change of stimulus intensity, flash-frequency or light adaptation; 3, latency and relative amplitude of off-wave. Under favorable chloralose anesthesia, the lateral locus shows longer recovery times and less negativity than the medial. But it is not depressed by narcosis or cautery of the latter. It fires independently of convulsant drugs applied at the medial locus, or of peripheral local stimulation of its topographic opposite.

The evidence suggests a separate (pulvinar ?) projection, resembling the second tactile field in area 1. The second visual area relates anatomically to 18, but seems hardly associative like the middle suprasylvian gyrus.

The effect of changes in food habit upon the respiratory exchange of intact and eviscerate rats. JAY TEPPERMAN (by invitation), J. R. BROBECK (by invitation) and C. N. H. LONG. *Dept. of Physiological Chemistry, Yale Univ. School of Medicine, New Haven, Conn.* Rats with symmetrical hypothalamic lesions pair-fed with normal littermates often consume their 24 hours' ration in one to three hours. Such animals exhibit abnormally high R.Q.'s during glucose absorption. Intact animals trained for a period of three to four weeks to eat their day's quota of food in three hours or less show quotients in the same range as those obtained in rats with hypothalamic lesions. This finding is in partial confirmation of the work of Werthessen (Am. J. Physiol. **120:** 458, 1937).

Preliminary studies showed that the R.Q. difference between "trained" and "untrained" animals persisted when the rats were anesthetized and given large amounts of glucose subcutaneously, and that, under these circumstances, the quotients of the "trained" rats were always substantially above unity.

Since the site of fat synthesis from carbohydrate in the animal body is not known, studies were made of the respiratory exchange of both intact and eviscerate "trained" rats given a plethora of glucose subcutaneously. 20 "trained" and 20 "untrained" eviscerate preparations showed quotients below 1.0. However, when "trained" eviscerate animals were given insulin, quotients over 1.10 were produced with great regularity, whereas "untrained" eviscerate animals tested under the same conditions exhibited quotients just below unity.

These studies suggest the possibility that both the liver and peripheral tissues may be sites of fat synthesis from carbohydrate in the rat. *This work was assisted by a grant from the John and Mary R. Markle Foundation.*

Quantitative studies on muscle and nerve regeneration. J. D. THOMSON (by invitation), B. LAZERE (by invitation) and H. M. HINES. *Dept. of Physiology, State Univ. of Iowa, Iowa City.* As part of an investigation concerning possible effects of various conditions upon the course of muscle and nerve regeneration, a study has been made of the extent and velocity of regeneration in the tibial nerve and gastrocnemius muscle of the albino rat. The tibial nerve was crushed in one limb at the level of the peroneal. The unoperated contralateral limb served as a control. At 12, 18, 21, 28, 35, 42, 56 and 84 days after operation animals were sacrificed for measurements of muscle weight, capacity to develop tension in response to direct and motor nerve stimulation and creatine concentration.

Following denervation there occurred a progressive decrease in muscle creatine, mass and strength as elicited through maximum direct stimulation and total absence of response to motor nerve stimulation. This continued until functional reinnervation occurred (2 to 3 weeks). Thereafter, atrophy was arrested and a progressive recovery of muscle creatine, mass and strength was noted. The rate of regeneration was rapid between the second and fifth week but showed a progressive decrease thereafter. At the end of 12 weeks, the regenerating muscles exhibited 85 to 90 per cent of normal mass and strength. Creatine concentration values closely paralleled the changes in muscle cell phase.

The age of the animals had a significant effect upon the extent and rate of regeneration. The faster rate of regeneration in the younger animals was due in part to a lesser distance required for axone regeneration and in part to increased rate of growth per se.

Spinal ganglia respiration. J. M. TOBIAS (by invitation), D. B. CLARK (by invitation) and R. W. GERARD. *Depts. of Physiology and Anatomy, Univ. of Chicago.* The improved capillary respirometer (Tobias and Gerard, 1940, and in press) permits ready measurement of respiration of single rat spinal ganglia (0.3 to 1 mgm., aver. 0.6) and so of fairly homogeneous neurone perikarya under various conditions. Oxygen uptake (cmm. /hr./ganglion) averages (49 thoracic ganglia) 0.94 + 0.024 at 75 mins. after decapitation, 0.80 + 0.024 at 165 mins. Neurone counts (obtained from corrected nucleoli counts in 100 serial sections) on 5 ganglia ranges from 1200 to 2700, average 2350; and respiration per neurone calculates at the high value of 4.5×10^{-5} cmm./hr. To allow for glial respiration, cat's filum terminale, containing almost pure glia, was measured. Oxygen usage (cmm. hr. moist gram) is: filum 1140, spinal root 400, peripheral nerve 260 at 80 mins.; 720, 350, and 210 at 180 mins.

Following section of the peripheral intercostal nerves on one side, respiration of ganglia on both sides increases (15 rats). The ganglia with cut processes reach a maximal respiration over 50% above that of control animals on the 4th day after section; the uncut ganglia show a similar but lesser maximum. Section of the dorsal root central to the ganglion led to similar changes in 2 experiments. Data will be presented on the influence of nerve stimulation on ganglion respiration.

Antithromboplastic activity of normal and hemophilic plasmas. LEANDRO M. TOCANTINS. *Division of Hematology, Dept. of Medicine, Jefferson Medical College, Philadelphia.* In plasma separated from normal blood *collected with especial precautions*, there is a substance which, on incubation with diluted aqueous extracts of brain tissue, reduces the thromboplastic action of these extracts. This inhibiting substance is exhaustible, shows a certain degree of species specificity, diminishes gradually on standing and rapidly when in contact with platelets in glass vessels, upon heating to 60° C for 5′, or upon dilution. It is usually absent in plasma from slowly collected, inadequately citrated blood. The antithromboplastic activity of hemophilic plasma is several times that of normal plasma and is preserved much longer on standing.

Antithromboplastin is probably the inhibitor chiefly responsible for maintenance of the fluidity of circulating blood. It prevents or delays the inception of clotting by inactivating any thromboplastin freed from blood and tissue cells, thus reducing the amount of available thromboplastin and retarding the transformation of prothrombin to thrombin. Exhaustion of the antithromboplastin renders possible the activation of prothrombin by any thromboplastin present in excess or subsequently released. The *essential* defect in hemophilia consists in the presence in the blood of antithromboplastin in excessive amounts, thus leading to the inactivation of most of the throm-

boplastin freed from the platelets when the blood is shed. This accounts for two *secondary* defects known to exist in hemophilia: the slow transformation of prothrombin into thrombin and the low thromboplastin content of citrated hemophilic plasma.

The human electromyogram in brief volitional twitches. J. E. P. TOMAN (by invitation) and ROBERT H. OSTER. *Dept. of Physiology, Univ. of Maryland School of Medicine, Baltimore, Md.* Human electromyograms during brief volitional twitches have been studied in six adult subjects in m. abductor pollicis brevis, m. flexor sublimis digitorum, m. biceps brachii, m. flexor carpii radialis, and m. extensor digitorum communis. It is confirmed that slight brief voluntary efforts may be accompanied by a single discharge of the motor unit whose activity is being recorded. (Gilson, A. S., and Mills, W. B. 1941. *Amer. J. Physiol.*, **133**: 658.) However, slightly stronger twitches are found to involve, not double or repetitive discharges of a single unit, but either 1, simultaneous discharges of two or more units or 2, serial discharges of two or more units, or 3, a combination of both 1 and 2.

Double discharges of a single unit are occasionally seen, but only with a latency of the same time order (about 100 msec.) as the frequency of response during a sustained contraction. With moderate or strong twitches, units may sometimes be seen to repeat one or more times, other units being also present in the electromyogram.

These observations are presented as further evidence of the importance of multifiber summation rather than wave summation in the ballistic type of voluntary muscular activity.

The hemodiluting capacity of rats with adrenocortical transplants. CAROLINE TUM-SUDEN and LELAND C. WYMAN. *Physiological Laby. of Boston Univ. School of Medicine and the Evans Memorial, Mass. Memorial Hospitals, Boston.* The tacit assumption that autoplastic adrenocortical transplants are functionally equivalent to the intact cortex is not true with respect to histamine poisoning and anaphylaxis (Wyman and tum-Suden, Endocrinology **21**: 587, 1937; tum-Suden, Am. J. Physiol. **108**: 416, 1934). Gaunt *et al.* (Am. J. Physiol. **120**: 532, 1937) demonstrated that adrenalectomized rats respond to intraperitoneal injections of isotonic glucose solution by greater and more prolonged hemoconcentration than do normals. The efficiency of transplants in the response to intraperitoneal glucose was therefore investigated. In 13 adrenalectomized rats with intramuscular transplants there was a rise in blood cell volume (cell opacity method) averaging 9.7 per cent of the initial value within thirty minutes, reaching a peak of 23.8 per cent in one to two hours, and being maintained from 7 to 12 hours before hemodilution set in. This course of events was confirmed by simultaneous hemoglobin determinations. In 10 normal rats the rise averaged 4 per cent initially, reached a peak of 10.5 per cent, and hemodilution began within 3 to 4 hours. In 15 adrenalectomized rats the rise averaged 7.2 per cent initially, and reached a peak of 28.5 per cent at death in around 3 hours. Hemodilution in one case began in 9 to 10 hours. Comparison of these three series confirms our conclusion that transplanted adrenocortical tissue, although able to maintain life and general health, is less efficient than the normal cortex in acute emergencies.

The acid-base equilibrium of the blood in exercise. EUGENE S. TURRELL (introduced by Sid Robinson). *Dept. of Physiology, Indiana University Medical School, Bloomington.* Blood samples drawn from human subjects in the basal state and after various intensities of exercise were analysed for O_2 and CO_2 capacity, lactic acid, plasma proteins, and serum pH. Comparison of increases in lactate concentration (Δ lactate) and decreases in CO_2 capacity (Δ CO_2 capacity) up to 4 mEq. per liter showed approximately equivalent changes. Beyond this point decreases in CO_2 capacity became progressively smaller than corresponding increases in lactate concentration. Base for neutralization of the lactic acid at low concentrations was obtained principally from bicarbonate. At the higher lactate concentrations hemoglobin and plasma proteins accounted for an increased fraction of this base. Decreases in serum pH of arterial blood (Δ pHs) varied directly with Δ lactate and Δ CO_2 capacity. The maximum blood lactate value measured was 22 mEq. per liter with a corresponding serum pH of 6.97.

The influence of hypophysectomy on the size, function, and ascorbic acid content of the adrenal gland of the rat. RICHARD TYSLOWITZ (by invitation) and E. B. ASTWOOD. *Depts. of Pharmacology and Medicine, Harvard Medical School, and the Medical Clinic of the Peter Bent Brigham Hospital, Boston.* The rate of regression in adrenal weight subsequent to hypophysectomy was measured in 276 male rats. The adrenal weight did not change during the first 24 hours after operation, rapidly decreased during the second 24 hours, and was reduced by 43.9 per cent after one week, by 53.5 per cent after 2 weeks, and by 58.8 per cent after 4 weeks. The regression rate may be expressed by a logarithmic-logistic function of the form

$$y = 9.27 - \frac{5.49x^2}{x^2 + 15.16},$$

wherein x is the number of days following hypophysectomy and y the adrenal weight in milligrams per square decimeter of body surface.

The functional capacity of the adrenal cortex was measured by the length of time the body temperature could be maintained above 30°C. in an environment of 0°C. The values obtained were: normal rats, 12 hours or more; 2–6 hours after hypophysectomy, 4 hours; 1, 2, and 3 days after hypophysectomy, 5.4, 4.4, and 2.9 hours respectively; 4–28 days after hypophysectomy the values fell between 1.5–2 hours and corresponded to those of adrenalectomized animals.

Chemical determinations of ascorbic acid in the adrenals showed an increased concentration of 29 per cent over the normal value 24 hours after hypophysectomy. Thereafter the concentration fell to below normal on the 4th day and reached 80.6 per cent on the 6th day, 66.4 per cent on the 14th day, and 61.5 per cent on the 56th day.

Thus hypophysectomy causes a rapid reduction of the functional capacity of the adrenal cortex while the regression in size and in ascorbic acid is slower and less marked.

The respiration of the spinal cord following 60 minutes of asphyxiation. A. VAN HARREVELD and D. B. TYLER (by invitation). *California Inst. of Technology, Pasadena.* The spinal cords of cats were asphyxiated for 60 minutes and by means of Warburg respirometers the oxygen uptake was determined of the region caudal of L 5. Determinations were made at the following intervals after asphyxiation: 50 minutes (this is the shortest period possible because of the time required to remove the tissue and to bring it in equilibrium in the bath), 4 hours, 7 hours, 12 hours, 2 days, 7 days and 14 days. The average oxygen uptake of the normal spinal cord was found to be 67 mm3. per 100 mgm. of wet tissue per hour. Fifty minutes after the end of the asphyxiation period it was 58 mm3., at 4 hours it was 56 mm3., 6 hours 48 mm3., 12 hours 38 mm3., 2 days 30 mm3., 7 days 32 mm3. and after 14 days it was 29 mm3. These results indicate that for a period of about 6 hours after asphyxiation the spinal cord is capable of considerable metabolic activity. From 48 hours on the respiration reaches a fairly constant level which is about 45 per cent of the normal. This offers an explanation in metabolic terms for the temporary return of reflex function of similarly asphyxiated spinal cords (J. Neurophysiol. 2: 101, 1939).

Acclimatization during discontinuous exposure to anoxia. EDWARD J. VAN LIERE and J. CLIFFORD STICKNEY (by invitation). *Dept. of Physiology, School of Medicine, West Virginia Univ., Morgantown.* There is still considerable controversy whether or not aviators show objective signs of acclimatization. There are but scanty reports in the literature which concern acclimatization during discontinuous exposure to anoxia. This question is of practical interest since aviators spend only a relatively small part of their time aloft. If they should become partially acclimatized, their "ceiling" would be raised and their efficiency increased.

To determine whether discontinuous exposure to anoxia produces acclimatization, four dogs were subjected daily (except Sundays) to a lowered barometric pressure by use of a low-pressure chamber. Three of the four dogs, which were maintained at a simulated altitude of 12,000 feet for about 6.5 hours a day, showed a slight rise in hemoglobin and erythrocyte count at the end of the third week. This was considered the threshold for the average dog. Two of the four dogs were subjected to a simulated altitude of 16,000 feet for about 8 hours a day and showed a pronounced rise in hemoglobin and erythrocyte count at the end of the third week. All four dogs at a simulated altitude of 18,000 feet showed a progressive rise in hemoglobin and erythrocyte count; at the end at the end of the seventeenth week there was an increase of 74 per cent and 84 per cent respectively.

It was concluded that discontinuous exposure to anoxia produces acclimatization; the degree, as measured by the hemoglobin determination and erythrocyte count, is directly proportional to the degree of anoxia and the length of time of exposure.

The thiamine requirement of the albino rat as related to the carbohydrate, protein and fat of the diet. WALTER W. WAINIO (introduced by E. B. Forbes). *Inst. of Animal Nutrition, State College, Pennsylvania.* The thiamine requirement of the adult, male, albino rat (Wistar Institute strain) has been assayed by a method which involves the maintainance of a normal excretion of pyruvic acid with measured amounts of thiamine.

The average, daily requirement of ten rats maintained in approximate energy equilibrium at 26°–30°C. on a diet containing 64 per cent sucrose, 20 per cent casein and 10 per cent hydrogenated cottonseed oil (plus cellulose flour, salts and vitamin supplements) was found to be 33 micrograms (coef. var. = 8 per cent). When nine of these rats were given a diet high in protein (64 per cent casein, 19.6 per cent sucrose, 10 per cent oil; representing a 9 per cent decrease in energy intake), they required an average of 20 micrograms of thiamine daily (coef. var. = 9 per cent). This demonstrates a 39 per cent reduction in the average requirement. The further administration of a diet high in fat (54 per cent hydrogenated cottonseed oil, 20 per cent casein and 16.6 per cent sucrose) when given isocalorically with the high protein diet lowered the average requirement of eight rats to 13 micrograms (coef. var. = 17 per cent). This value is 59 per cent lower than the requirement on the high carbohydrate diet and 32 per cent lower than the requirement on the high protein diet.

The calculation of requirement values in terms of the nutrients contained in the diets reveals that each gram of sucrose required the presence of 2.94 micrograms of thiamine in the diet and that each gram of casein and fat required the presence of 1.81 and 1.29 micrograms of thiamine, respectively.

Prophylactic treatment of experimental renal hypertension with renin. G. E. WAKERLIN, C. A. JOHNSON (by invitation), E. L. SMITH (by invitation), J. R. WEIR (by invitation), and G. A. OLANDER (by invitation). *Depts. of Physiology and Physiological Chemistry, Univ. of Illinois College of Medicine.* Three groups of four normotensive dogs were subjected to daily intramuscular injections of hog renin, dog renin, and heat-inactivated hog renin respectively for three months before and three months after constriction of the renal arteries by the Goldblatt technique. Following constriction, hypertension did not develop in two of the four dogs receiving hog renin, in two of the four animals given dog renin, and in one of the four dogs injected with inactivated hog renin. The other seven animals as well as two dogs treated with inactive human renin (renal extract prepared like renin but pressor inactive) developed the usual Goldblatt hypertension. Hypertension failed to develop in one dog treated prophylactically with rabbit renin. An untreated control group of fifteen dogs simultaneously subjected to constriction of the renal arteries by the same technique, all developed hypertension. Results from another control group of four dogs prophylactically treated with an extract of hog liver prepared after the manner of renin will be reported.

The serums of the dogs treated with hog renin or rabbit renin neutralized the acute pressor effect of renin (antirenin). The serums of the dogs treated with dog renin, inactivated hog renin, or inactive human renin, and the serums of the untreated control animals never showed antirenin.

The mechanism of these striking prophylactic effects of renin in experimental renal hypertension is not clear and requires further work for elucidation. Antirenin is probably not involved.

Respiratory effects upon the visual threshold. GEORGE WALD, HOWARD P. KRIEGER (by invitation) and HOWARD C. GOODMAN (by invitation). *Biological Labs. of Harvard Univ., Cambridge, Mass.* Respiratory effects upon the absolute threshold of vision were measured, using a 2° field centered 10° below a fixation point and exposed for flashes of 1/50 second. The field was viewed in most experiments through an artificial pupil 2 mm. in diameter. All measurements were performed on the completely dark adapted eye. The photochemical system was thus kept stationary, and the changes recorded may be assumed to have originated more centrally.

On increasing by 50 to 100 per cent the normal rate of breathing room air or 32-36 per cent oxygen, the visual threshold falls to about half its normal value within 5 to 10 minutes.

This change is due primarily to alkalosis induced by the hyperpnoea, and can be counteracted or reversed by adding CO_2 to the inspired gases. Normal or rapid breathing of 2 per cent CO_2 causes no change in threshold; with 5 per cent CO_2 the threshold is approximately doubled.

Breathing 10 per cent oxygen at the normal rate also approximately doubles the threshold. This effect is compensated in part by rapid breathing; usually in such experiments the threshold initially falls, then slowly rises to supranormal levels.

The opposed effects of respiratory alkalosis and acidosis upon visual threshold parallel the changes in cortical excitability observed in comparable circumstances (Dusser de Barenne et al.).

Effects of cochlear lesions on click responses in the auditory cortex of the cat. EDWARD M. WALZL (by invitation) and CLINTON N. WOOLSEY. *Depts. of Otology and Physiology, Johns Hopkins Univ., School of Medicine, Baltimore, Md.* Localized lesions in the cochlea which caused loss of response to a limited part of the frequency range as measured by cochlear potentials resulted in disappearance of cortical potentials to click stimulation in specific parts of the auditory area.

With lesions in the basal turn of the cochlea sufficient to cause complete loss of cochlear response to high tones, there was disappearance of cortical potentials from the anterior portion of the auditory area. With lesions in the apical turn of the cochlea, there was loss of cochlear response to low tones and disappearance of cortical potentials from the posterior portion of the auditory area.

These results indicate that there is spatial localization in the cerebral cortex for different regions of the sound spectrum.

The projection of the cochlea to the cerebral cortex indicated by these experiments agrees with the projection revealed by electrical stimulation of nerve fibers in local regions of the osseous spiral lamina, (Woolsey and Walzl. Proc. Am. Physiol. Soc., 1941).

Localization of the salivatory "points" in the lower brain stem of cat. S. C. WANG (introduced by Magnus I. Gregersen). *Dept. of Physiology, College of Physicians and Surgeons, Columbia Univ., New York City.* The portion of the brain stem caudal to the tentorium cerebelli has been explored for salivation in 22 cats, anesthetized with nembutal. The cerebellum was exposed and a bipolar electrode was introduced into the underlying structures with the aid of the Horsley-

Clarke stereotaxic instrument. The stimulating current was provided by a Harvard inductorium having one dry cell (1½ V) and a small rheostat (½ ohm) in the primary circuit, and with the secondary coil set at 13.5 cms. The ducts of the submaxillary or/and parotid glands were cannulated, and connected to a horizontally-placed, calibrated capillary tube for recording the secretion for each 15-second stimulus. The reactive region was subsequently determined histologically.

No significant flow of saliva was obtained by stimulating the pons or the caudal portion of the midbrain. In a plane through the facial genu and the facial nerve, the reactive region for the submaxillary gland was in the vicinity of the genu and also scattered in an area corresponding to the spinal trigeminal nucleus and tract. In another plane just caudal to the genu and through the facial nucleus, a copious flow of saliva was obtained by stimulating the dorsal border of the medial and the lateral reticular formation and also in an area lateral to the facial nucleus. At this and slightly caudal levels, stimulation brought about secretion from the parotid gland. The exact locations of the parotid "points" have not been determined histologically.

A study of dietary self-selection. JOHN WARKENTIN (introduced by A. C. Ivy). *Dept. of Physiology and Pharmacology, Northwestern Univ. Medical School, Chicago.* Our purpose was to determine the character of food choices in rats under conditions of experimental hypo- and hyper-thyroidism. Richter's method of dietary self-selection was used. The results of control studies warrant the following statements: 1. The majority of animals selected "successfully" from 6 different food cups which contained casein, dextrin, lard, brewer's yeast (cod-liver oil was added), agar, and Osborne and Mendel's salt mixture. "Successfully" means growth in the young or weight maintenance in older animals. 2. It is best to separate all the dietary constituents. For example, when yeast and salt were mixed together in equal parts, less than half the rats ate enough to maintain a normal growth curve. 3. There are great individual variations in selections among rats which do select a diet which supports growth. Furthermore, each rat may vary markedly in its selection during a period of more than 3 months. 4. Young rats fed the various constituents of the Osborne and Mendel diet separately (selective feeding) did as well as or better than rats given the ready-mixed Osborne and Mendel diet. 5. Rats kept on selective feeding did better if given agar to select than did controls without agar. 6. Age and dietary history affect selective feeding. Older rats did not prosper so well on selective feeding as did controls fed a ready-mixed diet.

Experimental hypo- or hyper-thyroidism did not significantly affect dietary self-selection.

The Pasteur effect in bone marrow. CHARLES O. WARREN. *Dept. of Anatomy, Cornell Univ. Medical College.* When rabbit bone marrow is exposed in vitro to lowered oxygen tensions, the critical level of oxygen tension below which the rate of respiration is decreased varies with the experimental procedure employed. When measured in the Warburg apparatus, with optimal rates of shaking, the critical oxygen tension for 0.5 mm. tissue slices is of the order of 20 vol. per cent O_2 (150 mm. Hg), and 2–5 vol. per cent O_2 (15–40 mm. Hg) for suspensions of marrow cells. With the dropping mercury electrode method, the critical tension for marrow suspensions is of the order of 0.8 per cent oxyben (6 mm. Hg). Only in the last method are the results free from distortion by diffusion and solubility factors, so that the latter figure appears to approximate the true critical oxygen tension.

In the present study and apparently in those cited in the literature, the Pasteur effect has been measured only under conditions in which the rate of respiration is determined by diffusion and/or solubility factors. Under such conditions, in the present instance, the changes in rates of respiration and glycolysis are essentially reciprocal. This reciprocal relationship has been demonstrated in tissue slices and in cell suspensions, in various media, and in normal and predominantly erythroid marrows. These findings afford no support for the concept of a Pasteur enzyme in this tissue, but on the other hand do not exclude such a possibility. The results also make it appear unlikely that an increase in glycolysis is the principal metabolic basis for the increased hematopoiesis observed at lowered oxygen tensions.

Influence of various salts on the toxicity of pontocaine hydrochloride. H. WASTL. *Dept. of Anatomy, Hahnemann Medical College, Philadelphia, Pa.* Studies on the toxic systemic effects of intramuscular injections of pontocaine hydrochloride into guinea pigs showed that it is even more toxic than Butyn sulfate. The dose of 25 mgm/kgm., leading to 100.0 per cent violent convulsions and 80.0 per cent mortality was used in comparative detoxicating experiments with sodium, potassium and calcium salts (halogen salts, levulinates, gluconates, lactates) in separate injections of them 18–20 minutes prior to pontocaine HCl injections as well as in mixture injections. The amount of salts administered was 400–600 mgm/kgm. In separate injections the halogen salts exert no protection and the other three groups of salts are merely able to lower in various degrees the mortality rate and violence of reaction, but rarely its incidence. In mixture injections the best protective results were obtained

with potassium levulinate and calcium levulinate, lowering the incidence of convulsions from 100.0 per cent to 25.0 per cent and 22.0 per cent respectively, with no mortality. Of these two potassium levulinate is locally non-irritant. Only these salt-pontocaine mixtures that were perfectly clear and showed no turbidity whatsoever were employed in this study. This made it impossible to employ some of the salts used in earlier studies with Procaine HCl and Butyn sulfate.

The part played by carotid and aortic body reflexes in respiratory control in unanesthetized dogs. J. G. WATT, P. R. DUMKE and J. H. COMROE, JR. (introduced by C. F. Schmidt). *Laby. of Pharmacology, Univ. of Pennsylvania.* Experiments were performed on 7 trained dogs to determine: *a*, if chemoreceptors are tonically active in the unanesthetized dog breathing room air; *b*, if the respiratory center of the unanesthetized dog deprived of carotid and aortic body reflexes is stimulated by oxygen-lack; *c*, if chemoreceptors are abnormally active under chloralose anesthesia.

Results: *a*, Some chemoreceptors are normally activated by arterial pO_2 present in unanesthetized dogs breathing room air, for inhalation of 100 per cent O_2 regularly led to immediate decrease in pulmonary ventilation. When inhalation was continued ventilation usually returned to normal in 1 to 3 minutes, but at 6 minutes arterial pCO_2 remained elevated (average increase 3.8 mm. Hg) in 5 out of 6 dogs. After chemoreceptor denervation O_2 inhalation no longer decreased respiration but either increased it or had no effect.

b. Low oxygen mixtures which previously increased breathing failed to do so after chemoreceptor denervation. A progressive depression of pulmonary ventilation was usually seen.

c. Chloralose (100 mgm./kilo intravenously) depressed respiratory minute volume (average reduction 36 per cent) and arterial pO_2 (average fall 25 mm. Hg) and raised arterial pCO_2 (average 5.2 mm. Hg). At the same time the thresholds to NaCN and to low O_2 were usually lowered and the period of respiratory depression following O_2 inhalation was prolonged. Chloralose anesthesia therefore exaggerates the importance of chemoreceptor reflexes.

Quinidine sulfate and ventricular fibrillation. RENÉ WÉGRIA and NEIL NICKERSON (by invitation). *Dept. of Physiology, Western Reserve Univ. Medical School, Cleveland, O.* The usefulness of quinidine sulfate as preventive of ventricular fibrillation has been investigated in two types of experiments performed on dogs under sodium barbital anesthesia.

I. Wiggers and Wégria have shown that the susceptibility of a ventricle to be thrown into fibrillation can be quantitated by determining its fibrillation threshold, i.e., the weakest short direct current able to initiate fibrillation when applied in late systole during the so-called "vulnerable period." By reviving the heart by the countershock method, changes in the fibrillation threshold can be repeatedly determined on the same animal.

Quinidine sulfate, when administered intravenously in doses of 2 mgr. per kilogram of animal, increases the fibrillation threshold significantly and for a significant length of time.

II. When 0.02 mgr. adrenalin per kilo is administered intravenously to dogs inhaling benzol, ventricular fibrillation results. In the same dog, two tests were generally made, i.e., the combination benzol-adrenalin fibrillated the ventricle twice. Each time the dog was revived by the counter-shock method. Then quinidine sulfate was administered intravenously in doses of 5 to 17 mgr. per kilo, after which the benzol-adrenalin test was repeated. Under such circumstances, quinidine sulfate protected the heart against the benzol-adrenalin combination. This protection lasted approximately two hours, sometimes more.

In conclusion, quinidine sulfate makes it more difficult to induce ventricular fibrillation by single electric shocks and prevents ventricular fibrillation induced by benzol-adrenalin. [*Supported by a grant from the John and Mary R. Markle Foundation.*]

Observations on the distribution of bromide between cerebrospinal fluid and serum. EVERETT G. WEIR. *Dept. of Physiology, College of Medicine, Howard Univ., Washington, D. C.* Experiments dealing with the distribution of bromide ion between C.S.F. and serum were conducted *a*, to determine the equilibration rate between the C.S.F. and serum following intravenous injection of NaBr; *b*, to determine the effect, if any, of varying serum bromide levels upon the bromide distribution ratio, and *c*, to compare the effects of intravenous, and intracisternal administrations of NaBr.

Dogs were used exclusively in this study. C.S.F. was obtained by cisternal puncture. Bromide was determined potentiometrically using the silver electrode.

The results indicate *a*, that equilibrium between C.S.F. and serum is attained only after several hours; *b*, the distribution ratio appears to be related inversely to the serum bromide concentration, and *c*, intracisternal administration of NaBr alters (slows) the rate at which equilibrium is established or alters the final value of the distribution ratio.

The penetration of sugars in the aqueous humour. C. B. WELD and W. H. FEINDEL (by invitation). *Dalhousie Univ., Halifax, N. S.* The concentration of sugar in the blood of dogs was raised to two or three times the normal value by rapid

intravenous injection and kept at approximately that level by continuous injection. Xylose, glucose, sucrose and raffinose were used and determined by the Hagedorn and Jenson method. The aqueous from one eye was taken before beginning the injection and the other aqueous obtained at a predetermined time during the course of the injection of the sugar. Thus in one animal only one point on a time curve was obtained.

Xylose and glucose enter the eye almost immediately, the xylose more rapidly than the glucose. The xylose gave a decrease in concentration between aqueous and blood of some 25 per cent within 3 minutes while the glucose requires 10 minutes to show the same change. With glucose the penetration is more gradual, the concentration gradient falling by only 10 per cent within 2 hours. In the case of raffinose it is doubtful if there is any penetration at all.

The increase in concentration of glucose in the aqueous apparently never reaches a value of more than 50 per cent of the increase in the blood, even after 3 hours, suggesting that the eye has some mechanism to limit its concentration in the aqueous. This phenomenon is not observed in the case of xylose.

Despite a considerable difficulty in maintaining the blood sugar at a reasonably constant level and despite a difference in animals, it is abundantly clear that the rate of penetration of sugar from the blood into the eye is directly related to the molecular size of the sugar.

A portable apparatus for measuring blood flow in the extremities. HERBERT S. WELLS. *Dept. of Physiology and Pharmacology, Bowman Gray School of Medicine of Wake Forest College, Winston-Salem, N. C.* (Demonstration). The apparatus, which is pocket sized and portable, allows measurement of blood flow by the principle of Brodie. Following venous occlusion, the initial rate of swelling of the limb is measured by displacement of air from a thin rubber bag, 2.7 cm. wide, encircling the limb and held snugly against the skin beneath an adjustable cuff marked to indicate the volume enclosed. Inflation of the cuff bag to a constant pressure of 6 cm. water is effected by connection to a second bag, on which a lead weight is floated. Air passes between these bags either directly through a bypass tube, or (when the bypass is clamped) through a horizontal 1 cc. pipette containing a drop of water, movement of which may be timed for measurement of the rate of swelling of the limb segment under the cuff.

Volume changes of 0.1 to 0.2 cc. may be timed with a stopwatch or by counting swings of the half seconds pendulum. Reliability of the method for measuring the resting blood flow is indicated by analysis of simultaneous arm plethysmograph and "cuff method" tracings of the swelling of the limb. [*Aided by a grant of the Council on Physical Therapy of the American Medical Association.*]

Observations on hemorrhagic shock. J. M. WERLE (by invitation), R. S. COSBY (by invitation) and C. J. WIGGERS. *Dept. of Physiology, Western Reserve Univ. Medical School, Cleveland, O.* (Read by title.) In dogs, anesthetized with barbital or chloralosane, we reinvestigated whether hemorrhage *per se* can induce shock and, if so, the conditions for its production, the possibility of standardizing the procedure, the criteria for its recognition and the nature of the eventual failure.

Conclusions. Hemorrhage sufficient to maintain an effective period of marked hypotension can produce shock, for 1, the condition is benefited only temporarily by reinfusion of all withdrawn blood, and 2, it is generally accompanied by edema, congestion and hemorrhage of the duodenal and jejunal mucosa with excessive secretions and blood in their lumen.

The degree and duration of the hypotension necessary to induce shock vary in different dogs; apparently an elusive "resistance factor" interferes with experimental standardization of the procedure.

Irreversibility after generous infusions, admittedly unsatisfactory as a practical criterion of shock, is the only reliable one. Arterial pressure pulses offer no distinctive characteristics by which recovery or failure can be predicted.

In the majority of experiments progressive circulatory failure after reinfusion developed without the anticipated decline of effective venous pressures. Maintenance of adequate venous pressure was not a guarantee against development of shock. Tendency toward slight hemoconcentration after reinfusion occurred consistently regardless of subsequent recovery or failure. Induction of greater hemoconcentration by plasmapheresis had no accelerating effect on production of shock.

Circulatory failure after infusion either occurred gradually or quite suddenly. Supervention of alternans during the early cardiac acceleration, poor response to rapid infusion, maintenance of venous pressures and progressive cardiac slowing suggest cardiac impairment. [*Supported by a grant from the Commonwealth Fund.*]

Estrogen excretion associated with dysmenorrhea. NICHOLAS T. WERTHESSEN (introduced by G. Pincus). *Endocrine Laby. of the Boston Dispensary and the Pratt Diagnostic Hospital, Boston, Mass.* Fifty cases of dysmenorrhea were examined during a forty-eight hour period for the rate of estrogen excretion between the 16th and 20th days of the menstrual cycle. Twelve controls showing normal cycles completely free from pain were used to determine the normal rate of excre-

tion during this period. Two ovariectomized women possessing uteri were also used in the study.

It was found that in all cases the estrogen excretion of the subjects showing painful menses was less than that of the normal individuals and in most instances approximated that of the ovariectomized patients.

If it is assumed that the excretion rate is an index of the secretion rate, then these data are in direct contrast to those of Sturgis and Albright who found a normal endometrial pattern in their dysmenorrheic patients. The assumption leads to the hypothesis that ovulation or endometrial biopsy per se is not an index of normal secretory function by the ovary.

Causal mechanisms in the cardiac effects of high oxygen pressures. W. V. WHITEHORN (by invitation) and J. W. BEAN. *Dept. of Physiology, Univ. of Michigan.* The possible mechanisms in the bradycardia and slowing of auriculoventricular conduction in decerebrate dogs exposed to 5 atmospheres of O_2 were investigated. With vagi intact, these effects usually appeared after 1 to 1½ hours of exposure. In vagotomized animals, however, heart rate and conduction time changed little until after 2 to 3 hours. The subsequent pronounced slowing in rate and conduction under high O_2 might suggest involvement of sympathetic supply, direct action of O_2 on cardiac mechanisms, or the intermediation of some humoral mechanism. The reactions of stellatectomized animals were essentially similar to those of animals with intact nerve supply, except for a more gradual decline in heart rate. Bilateral stellatectomy and vagotomy together did not prevent the bradycardia or lengthening of P–R interval.

The experimental finding that changes similar to those induced by high O_2 are observed in animals breathing high CO_2 or low O_2 mixtures suggests that the cardiac effects of high O_2 may be attributable to an accumulation of CO_2 in the tissues and an anoxic effect of respiratory enzyme poisoning.

Incidental to the effects of vagotomy, it was observed that vagal cold block in decerebrate dogs frequently induced a *slowing* of the heart. The explanation of this phenomenon is not clear, but a combination of various factors such as low vagal tone, vagal accelerator mechanisms, absence of centers above the pons, and the exceedingly violent respiratory response to vagal block in the exceedingly violent respiratory response to vagal in the decerebrate dog may play a part.

Cardiac and peripheral determinants of hemorrhagic shock.[1] C. J. WIGGERS and (by invitation) JACOB M. WERLE and P. C. SHEA. *Dept. of Physiology, Western Reserve Univ. Medical School, Cleveland, O.* Dogs under morphine-barbital were bled until a marked state of hypotension was maintained for several hours. At the end of such a period the withdrawn blood (heparinized) was reinjected. A state of shock was considered to exist when such reinfusion failed to maintain arterial pressures for at least three hours and the upper intestines showed hemorrhagic changes at autopsy.

Cardiometer curves were recorded optically with aortic and venous pressures, and simultaneously on a kymograph as well. Changes in cardiac behavior were assessed from critically evaluated optical volume curves. Total peripheral resistance (TPR) was calculated from $\frac{\text{mean pressure} \times 1332}{\text{cardiac output/sec}}$ by use of calibrated drum records.

Experiments on 11 dogs consistently showed decreases in stroke and minute volumes during post-hemorrhagic hypotension and post-infusion failure, but the manner in which such reduction was occasioned differed. In one group, decrease in stroke was accompanied by decreasing venous pressures, in another it developed despite an elevation. Analyses of volume curves indicate that the capacity of the ventricles to respond to a given venous pressure (stretch) is reduced and that such hypodynamic action is masked when venous pressures decline concurrently. Prolonged reduction in coronary flow during severe hypotension is suggested as the cause.

Total peripheral resistance generally increased during the post-hemorrhagic hypotension and post-infusion regardless of whether irreversible failure developed or not. Augmented TRP seems to retard rather than hasten circulatory failure, for in animals in which TPR remained essentially unchanged or decreased this facilitated development of hemorrhagic shock.

The effect of gross bilateral lesions of the spinal cord (C-8 to T-1) on the effective venous pressure in the anesthetized cat. HAROLD C. WIGGERS, G. H. GLASER (by invitation), K. DES. CANAVARRO (by invitation) and A. E. TREAT (by invitation). *College of Physicians and Surgeons, Columbia Univ., New York City.* Intra-atrial (IAP), intrathoracic (ITP) and carotid pulse pressures were registered simultaneously by optical membrane manometers and the effective venous pressures (EVP = IAP minus ITP) were determined in twelve cats anesthetized with nembutal. Average control IAP, ITP and EVP values were respectively 40, −39, and 79 mm. of saline during expiration; −21, −95 and 74 during inspiration. Only the expiratory values will be reported.

Spinal cord lesions were produced by a new procedure (Treat, Am. J. Physiol. **134:** 310, 1941). The cord was completely severed in seven animals; in these the circulatory changes were uni-

form in direction. Thirty and sixty minutes respectively after transection, the average values were as follows: IAP, 59 and 63 mm. of saline; ITP, −45 and −47; EVP, 104 and 112. This EVP elevation is apparently not respiratory in origin.

In five animals the cord lesions were extensive but the degree of transection was indeterminable upon gross inspection. Histological studies of the injured cords are now in progress. The large reduction in heart rate and arterial blood pressure in these as in the above seven animals suggests that the functional continuity of the ventro-lateral columns had been abolished. The IAP and EVP remained within the normal range or fell slightly. In this respect, however, it is important to note that the control EVP values in these animals were very high and that the ITP was markedly elevated following cord injury.

In all instances the pulse pressures either failed to change or increased insignificantly. There was no evidence of cardiac failure.

Further observations on the lingual pseudomotor phenomenon. C. WIGGINTON (by invitation) and K. B. CORBIN. *Division of Anatomy, Univ. of Tennessee College of Medicine.* (Read by title.) The roots of the right hypoglossal nerve were sectioned intracranially in 34 cats. Fourteen days later using Nembutal, the chorda-lingual, hypoglossal and cervical sympathetic nerves were exposed, and the tip of the tongue connected by thread to a muscle lever writing on kymograph. Stimuli at 30 per sec. were from Harvard inductorium at 5 to 7 cm.

Stimulation of the right chorda-lingual produces, latency about 2 sec., a contracture, reaching height in about 8 sec. A decrease in tension during stimulation is frequently seen after 5 to 10 sec. of maximal tension, with gradual return to pre-stimulation level during, or about 5 sec. after, stimulation. This contracture is accompanied by flushing of the tongue and by marked increase in rate of fibrillation. Following stimulation there is blanching and an almost complete cessation of fibrillation for 15 sec. Stimulation of the intramedullary portion of the seventh nerve and surrounding reticular matter produces almost identical results.

Stimulation of the right cervical sympathetic trunk causes a shortening of tongue after a long latent period of 5 to 10 sec., reaching its height in about 35 sec., and gradually declining for 40 sec. following a 30 sec. stimulus. This response is almost entirely abolished by cutting right hypoglossal extracranially and can then be reobtained on stimulation of distal end of cut hypoglossal. Accompanying cervical sympathetic trunk stimulation is marked blanching; no effect on fibrillation is seen during or after stimulation.

The estimation of the sodium associated with extracellular acid groups of muscle. W. S. WILDE (introduced by R. Ashman). *School of Medicine, Louisiana State Univ., New Orleans.* Ionic exchange of Ca and Na at high Ca/Na ratios will occur in excised turtle ventricle without evidence that Ca has reached equilibrium in the fiber water in any manner to suggest permeability. Thus Ca and no doubt Ba because of ionic diameter and low dissociation are suitable agents for "precipitating" acid radicles on fiber surfaces or intercellular matrix. The quantity of Ca bound on these groups will depend on the activity of Ca^{++}; as against that of $Mg,^{++}$; Na^+, K^+, H^+. the chief being Na^+. These activities in the interstitial fluid of frog sartorius are approximately that of the special fluid of Conway and Boyle (J. Physiol. 100, 3). The difference in Ca content of 6 sartorii soaked 2 hours in this fluid and their mates soaked in this fluid minus Na but with complementary glucose is 3.25 m.-equiv. per Kg. of fresh tissue. If Ca alone is increased in both solutions from 1.08 mM through values up to 12.96 mM intermediate differences occur up to 10.8 m.-eq. Further increase in Ca concentration imposes hypertonicity. The overall change from freshly excised muscle to muscle soaked in 12.9 mM Ca with no Na amounts to 22.0 m.-eq. Thus a conservative minimum for extracellular bound Na is 3.25 m.-eq. with a maximum that is indeterminate because of the unknown effect of the ionic strength of the different cations. Direct Na determinations place so-called intracellular Na of freshly excised frog muscle at 10.0 m.-eq./Kg.

Combination of hypoxic and hypercapnic stimulation at the carotid body. CLAUDE V. WINDER. *Department of Physiology, Univ. of Michigan Medical School, Ann Arbor.* The isolated carotid body regions of anesthetized dogs were perfused by means of a continuous artificial circuit with continuous gaseous equilibration of the basal perfusion fluid (heparinized blood and Locke's solution). Brief substitutions of experimental fluids equilibrated with gases high in CO_2, low in O_2, or a combination of the two, yielded reflex respiratory responses such that the combination effect on minute-volume of CO_2 and low O_2 was approximately equal to the sum of their individual separate effects.

The results are interpreted in terms of the theory that intracellular cH is a factor in the control of chemoreceptor activity.

The chemoreceptors are considered as one of several probable sites for mutual facilitation of hypoxic and hypercapnic stimulation of respiration.

Some consequences of intrauterine asphyxiation in the guinea pig. W. F. WINDLE and R. F. BECKER. *Northwestern Univ. Medical School.* (Read by title.) Anoxia induced and resuscitation accom-

plished in guinea pigs at term, as described by Becker and Windle in this volume, led to symptoms of brain lesions after birth. Animals were unable to right themselves and exhibited recurrent extensor hypertonus, opisthotonus, choreiform movements and running movements for an hour or more. For several hours to several days they were tremorous and ataxic, tending to fall or turn to one side. Some hyperesthesia was observed. Slight incoordination persisted a week or more. Few animals vocalized normally; some were unable to suck or swallow and lost weight in spite of artificial feeding. The startle response to sound was usually deficient and a hyposensitivity to cutaneous stimulation was manifested. One animal had motor and sensory loss in the hind legs and the bladder symptoms of a complete cord transection.

A few asphyxiated guinea pigs died soon after resuscitation; at autopsy small hemorrhages were found in various organs. All those surviving the initial shock recovered so completely that it was usually impossible to detect any difference between asphyxiated and control specimens by one month. Ability to learn problems on a simple maze like that used for the crawfish (Yerkes) was tested at one to three months. Some asphyxiated animals were incapable of learning the simplest problems. Others were less able to adapt to new situations than their littermates, failed to profit by past experiences, and made associations with difficulty.

Spontaneous potassium poisoning in the adrenalectomized animal. ALEXANDER W. WINKLER, PAUL K. SMITH (by invitation) and HEBBEL E. HOFF. *Dept. of Internal Medicine, and Labs. of Pharmacology, and Physiology, Yale Univ. School of Medicine, New Haven, Conn.* Dogs were subjected to bilateral adrenalectomy and were subsequently maintained on extract and salt until recovery. Salt and extract were then discontinued, although food (Bovex) and water were offered. Animals were observed until death with attention to changes in the electrocardiogram and serum potassium. Animals ate and drank little, and frequently required gavage with 20 per cent dextrose to prevent hypoglycemia.

In certain animals the serum potassium, after remaining only slightly elevated for some days after withdrawal of salt and extract, suddenly rose progressively over an 8 to 24 hour period. This rise was accompanied by characteristic electrocardiographic changes associated with elevated serum potassium. Within a few hours after disappearance of the P waves such animals died of cardiac arrest following progressive intraventricular block, characteristic of potassium poisoning, with concentrations of potassium in the serum of 10 to 15 mM per liter at death. Cardiac arrest preceded cessation of respiration.

It is concluded that one immediate cause of death in adrenalectomized animals may be cardiac arrest due to spontaneous elevation of the serum potassium. This is not, of course, the sole mode of death, since adrenalectomized animals may die without evidence of potassium poisoning.

Factors influencing the diuretic effect of progesterone and desoxycorticosterone acetate. HELEN WINTER (by invitation) and HANS SELYE. *Dept. of Anatomy, McGill Univ., Montreal, Canada.* Experiments in albino rats confirm the fact that both progesterone and desoxycorticosterone acetate (D.C.A.) increase the daily urine output. In rats receiving neither food nor water D.C.A. proves somewhat more effective in this respect than progesterone. The same is true in animals receiving iso-, or hypertonic NaCl solutions by mouth, while in the case of oral administration of distilled water the diuresis produced in rats chronically treated with progesterone may be as good or even better than that obtained in animals similarly treated with D.C.A.

The urinary chloride elimination in rats receiving neither food nor water or only distilled water is increased by progesterone, but not significantly changed by D.C.A. conversely after oral administration of iso-, or hypertonic NaCl solutions the urinary chloride elimination is increased and accelerated by D.C.A. but not by progesterone.

These experiments indicate that salt and water intake markedly influence the diuretic effect of the above mentioned steroid hormones as well as their action on chloride excretion, a fact which may perhaps partly explain the contradictory results concerning D.C.A. published by previous workers (H. Selye and L. Bassett, Proc. Soc. exper. Biol. Am. Med. **45**: 272, 1940; E. L. Corey and S. W. Britton, Am. J. Physiol. **133**: 511, 1941). Verzar (Schweiz. med. Wchnschr. **70**: 1229, 1940) believes that the overdosage symptoms noted in patients receiving D.C.A. and salt therapy are due to the fact that D.C.A. administered simultaneously with NaCl delays excretion of the latter. Our observations indicate that at least in the rat D.C.A. causes no such delay.

The relative retentions of infused chloride, urea, and water. A. V. WOLF (introduced by E. F. Adolph). *Dept. of Physiology, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.* Since the clearances of threshold and of non-threshold substances have different kinetic significances, a uniform method of analysing such diverse types of renal excretion was devised.

Dogs with exteriorized ureters from which urine was continuously collected were intravenously infused for 7 hours with solutions of NaCl — urea in various concentrations, at rates of about

2.3 cc./min. From inputs and outputs, *loads* of solutes and solvent were computed, as well as *velocity constants* of excretion (minute rate of excretion/load), *retention concentrations* (load of solute/load of water) uncorrected for insensible water loss, and *relative retentions* (retention concentration/infused fluid concentration).

It was found that only 0.6 per cent and 1.7 per cent NaCl were retained in the same concentration as infused; the velocity constants of Cl and of water became equal at these infusion concentrations and the relative retention was 1. Such solutions were "isorrheic" since they could be excreted at the same constant rate as infused without relative retention of solute or solvent, when the load became great enough.

The velocity constant of Cl was a linear function of its infused concentration. That of urea was independent of its own infused concentration. It is suggested that the rate of excretion of Cl varies not only with load but also with the departure from normal of the relative sizes of chloride and non-chloride volumes of distribution.

By this method of analysis the renal excretions of water and of diverse solutes find expression in common terms.

Studies on pain. Pain mechanisms in the stomach and observations on the sensibility of the gastric mucosa. STEWART WOLF (by invitation) and HAROLD G. WOLFF. *Depts. of Medicine and Psychiatry of the New York Hospital and Cornell Univ. Medical College, New York City.* Observations were made on a subject with a permanent gastric fistula 4 cm. in diameter. Various sensory stimuli were applied to the wall within the cavity of the stomach as well as to a redundant fold of mucosa protruding through the stoma. It was found that:

1. Pain resulted from distension of the muscular and peritoneal coats of the stomach either from pressure of a blunt object applied to the mucosa, inflating a balloon within the viscus, or from the force exerted by an unusually vigorous contraction of the stomach wall. The strength of stimulus necessary to induce pain by any of these methods applied gradually was equivalent to the pressure exerted by a 30 mm. column of mercury. Sudden stimuli of weaker intensity caused pain.

2. The size of the area stimulated ranged from ½ sq. cm. to 100 sq. cm. Neither threshold nor intensity of the pain experienced, however, was appreciably altered by varying the size of the area stimulated.

3. Pricking or pinching the stomach mucosa, or the application of faradic stimuli or strong acid failed to induce pain unless the mucous membrane was congested and oedematous.

4. Sensation of touch was absent in the gastric mucosa.

5. Pressure sensation was appreciated in the stomach and roughly localized. 2 pressure points could be distinguished at distances of more than 9 cm.

6. A warm stimulus above 40°C applied to the stomach mucosa was appreciated, as was a cold stimulus below 18°C. Between these limits no sensation was felt. Outside the limits temperature differences of 3°C or more were distinguished.

Effects of elevated serum potassium levels on the heart-lung preparation. E. H. WOOD and G. K. MOE (introduced by M. B. Visscher). *Dept. of Physiology, Univ. of Minnesota.* Various studies show that changes in potassium and calcium concentrations and ratios may under certain conditions produce digitalis like effects or modify the action of cardiokinetic glycosides. Investigations in this laboratory have demonstrated that the therapeutic and toxic actions of digitalis glycosides on the heart-lung preparation are uniformly unaccompanied by an increase in serum potassium content.

Isotonic KCl (1.23 per cent) was injected into the left auricular cavity at rates such as to produce increases in serum potassium concentration comparable to those observed in the efficiency increase period of digitalis action. Either no effect or increases in the rate of cardiac failure occurred in every instance. When the KCl injection is continued atrial dropped beats occur which progress to complete atrial asystole. Atrial asystole soon becomes irreversible by means of stopping the KCl injection, the ventricular rate becomes progressively slower and the preparation ends with the onset of ventricular asystole. Serum potassium concentrations at which atrial and ventricular asystole occur are uniform and average 34.5 and 38.3 mgm. per cent respectively. The corresponding plasma potassium levels which produce similar effects in the intact dog are much higher: 39.4 and 59.0 mgm. per cent respectively (Winkler et al., 1938). Suitable injections of 2 per cent $CaCl_2$ are capable of restoring the heart rate and work output of potassium poisoned heart-lung preparations to normal control values in the presence of serum potassium levels 10 mgm. per cent higher than the level which ordinarily produces ventricular asystole.

Potassium, sodium and chloride analyses of the ventricles of potassium poisoned heart-lung preparations show intracellular potassium concentrations of these hearts to be increased about 17 per cent over average normal and control values. Preliminary observations indicate that this increase of cardiac intracellular potassium content can be abolished by suitable increases in serum calcium levels. [Present address of authors: Department of Pharmacology, Harvard Medical School.]

Fibrillation in the unilaterally denervated dia-

phragm of the rat. CLINTON N. WOOLSEY, GEORGE J. HAYES (by invitation) and STANTON E. DEELEY (by invitation). *Johns Hopkins Univ., School of Medicine, Baltimore, Md.* (Motion picture demonstration.) The diaphragm of the rat is a structure in which it is particularly easy to observe fibrillation in denervated striated muscle. In this short film the ceaseless activity which characterizes such muscle is clearly shown in natural color. [*Sponsored by the National Foundation for Infantile Paralysis.*]

The effect of serum on the survival and revival of brain oxidations. JOSEPH WORTIS (introduced by I. M. Korr). *Dept. of Psychiatry, New York Univ. College of Medicine.* Experience with hyperglycemic or protracted insulin coma suggested the possibility that brain oxidations may be suspended in vivo through the depletion or destruction of certain substances—other than oxygen or glucose—contained in whole blood. Inasmuch as clinical experience has shown that blood transfusions serve to alleviate irreversible coma, in vitro studies were undertaken to test the effect of certain blood constituents on the survival and revival of brain oxidations. Minced brain of adult white rat was used. The oxidation curve of minced brain in plain Ringer phosphate solution buffered at pH. 7.38 flattened off in approximately six hours. The addition of glucose to the suspension medium did not revive brain oxidations after this period. There was, however, a small but significant revival of oxidations upon the addition of serum and glucose to the suspension medium. In a similar suspension medium containing 200 mgm. per cent of glucose, there was an abrupt flattening of the oxidation curve ar about eight hours. This cessation of oxidative activity was not due to lack of substrate, since glucose determinations revealed a substantial excess of glucose still present in the immersion fluid after oxidations had practically ceased; nor could it be attributed to the production of any inhibiting substance, since oxidations were not revived after the tissues had been washed in Ringer's solution and placed in a fresh suspension medium. This abrupt flattening did not occur if a small amount of serum was present in the suspension fluid, but oxidations continued at a practically constant rate for the duration of the experiment (12 hrs.).

Sensitization of intestinal smooth muscle to adrenalin by sympathetic denervation. W. B. YOUMANS, A. I. KARSTENS (by invitation) and K. W. AUMANN (by invitation). *Dept. of Physiology, Univ. of Oregon Medical School, Portland.* (Read by title.) In previous studies it has been shown that extrinsically denervated intestinal segments in the form of Thiry fistulae in unanesthetized dogs are hypersensitive to the inhibitory action of adrenalin and various other phenylethylamine derivatives. Further studies by similar recording techniques have now been completed to determine to what extent the sympathetic denervation is responsible for the sensitization.

Four dogs each having two Thiry fistulae of the upper jefunum were vagotomized above the diaphragm to eliminate the vagal innervation of each loop, and one of the loops was denervated locally by destroying the nerves in the mesenteric pedicle. The completeness of the denervation was indicated by the lack of a pain response to severe intestinal distention. The two loops prepared in this way differ from each other in that one loop has its sympathetic innervation intact while the other is postganglionically sympathetically denervated.

In each of the four dogs the sympathetically denervated loop of intestine showed a greater degree of inhibition of motility during a constant intravenous adrenalin injection than was observed in a simultaneous record from the sympathetically innervated loop. The hypersensitivity was present as soon after denervation as records could be obtained (two days) and persisted for months.

In animals having the pre-aortic ganglia decentralized, in addition to the operations mentioned above, the postganglionically denervated loop was found to be considerably more sensitive to adrenalin than the preganglionically denervated loop.

THE AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS

Thirty-Sixth Annual Meeting

Boston, Mass., March 31, April 1, 2, 3, 4, 1942

(For possible corrections in any of the following abstracts see the next issue)

Availability of formylglycine, acetylglycine and propionylglycine for the synthesis of hippuric acid of the rabbit. Lynn De Forrest Abbott, Jr. *Dept. of Biochemistry, Medical College of Virginia, Richmond.* Some of the N-acyl and N-alkyl derivatives of certain essential amino acids can replace the unsubstituted amino acids for purposes of growth in the diet of the young white rat. This method of study, however, cannot be used to investigate the derivatives of those amino acids which are not necessary in the diet for purposes of growth.

Glycine, when given with benzoic acid to rabbits, increases greatly the rate of excretion of hippuric acid as measured by the urinary excretion in the first six hours after administration. No other amino acid studied affects the rate of excretion in this way. The effect on rate of excretion of hippuric acid when various N-alkyl glycine derivatives were administered with benzoic acid to rabbits has been reported previously. In the present study this method has been used to investigate some N-acyl derivatives of glycine. Results of experiments with the formyl, acetyl and propionyl derivatives of glycine will be presented. These derivatives of glycine when administered with benzoic acid to rabbits increased the rate of excretion of hippuric acid. The availability of these compounds for the synthesis of hippuric acid is interpreted as evidence of their conversion to glycine by the rabbit.

Studies of experimental amino acid deficiency in man. II. Nitrogen and sulfur partition in the urine. Anthony A. Albanese, Charlotte Kajdi, and Dorothy M. Wangerin. *Dept. of Pediatrics, Johns Hopkins University, Baltimore, Md.* In four *tryptophane deficient subjects* no significant variations were observed in the output of urea, creatinine, and ammonia. The excretion of phenols gradually decreased during the first two weeks of the experimental period. In two subjects who were then returned to a control diet the phenol excretion returned to normal within 10 days. Two other subjects were continued on the experimental diet for an additional three weeks; during this time there occurred a gradual rise in phenol excretion with a return to the normal after the termination of the experimental period.

The excretion of cystine and methionine showed no consistent variations during the period of deficiency. There was, however, a marked and sustained increase in SO_4^- excretion during the period of tryptophane deficiency. The SO_4^-/N ratio in the urine was likewise increased throughout this period.

In five *lysine deficient subjects* no characteristic changes were observed in the excretion of urea, creatinine, or ammonia. The phenol excretion showed a slight increase of questionable significance. Neither the excretion of cystine nor methionine showed significant fluctuations but the SO_4^- output was markedly increased, as was the SO_4^-/N ratio. These changes returned to normal in the succeeding control period.

In three subjects with *arginine deficiency* there was observed a marked decrease in ammonia output in the deficient period which returned to normal in the subsequent control period. No changes were observed in the excretion of urea, creatinine and phenols. The excretion of cystine, methionine and of sulfate likewise remained unchanged during the 10 day deficiency period.

The enzymatic conversion of quinine in vitro by tissues of the rat. Carl E. Anderson and James C. Andrews. *Dept. of Biological Chemistry, School of Medicine, Univ. of North Carolina, Chapel Hill.* Lipkin, in 1919, indicated that quinine is "enzymatically destroyed" by minced tissues of the guinea-pig, rabbit, sheep, and ox. We have confirmed this work for some of the tissues of the rat. Minced liver, kidney, brain, and lung destroy quinine when incubated with these tissues at 40°C. for 24 hours. The high degree of activity of the liver prompted attempts to isolate an enzyme preparation from this organ. By extraction with 50 per cent alcohol and precipitation at 70 per cent we have obtained a crude, active preparation from the livers of rats. Aliquots of this preparation when incubated at 40°C. for 24 hours with 10 ml. of a solution containing 4.91 mg. of quinine, as free base, per liter resulted in losses of from 20 to 60 per cent of the original quinine. The preparation contained considerable amounts of denatured protein. This may account for the variation in the rate of destruction. At ice box temperature (+5°C.) there was a decrease in activity. In one controlled experiment a loss of 24.5 per cent of the original quinine (as free base) was obtained as against a loss of 38.7 per cent at 40°C. 0.8 per cent NaF, and exclusion of air by covering the reaction mixture with paraffin oil, inhibited the activity of the enzyme. Chloroform did not inhibit its activity.

The enzymztic conversion of quinine in vivo in the

rat. JAMES C. ANDREWS and CARL E. ANDERSON. *Dept. of Biological Chemistry, School of Medicine, Univ. of North Carolina, Chapel Hill.* Several investigators have indicated that quinine is "destroyed" by perfusing the livers of dogs and cats. Lipkin, by the separation of a crude enzyme product from the liver of the guinea pig, showed that, *in vitro*, the "destruction" was enzymatic. The result has been confirmed in this laboratory for the tissues of the rat. Since Kirschbaum showed that quinine has little or no anti-malarial activity in blood cultures of the parasite, a knowledge of the conversion of quinine *in vivo* is of vital importance in understanding its anti-malarial activity, and also as a clue to the synthesis of new, much-needed, anti-malarial compounds.

We found that, in young adult rats with normal livers, the excretion of quinine in the urine varied from 5 to 14 per cent of that introduced orally. In the same rats, after removal of from 30 to 50 per cent of the liver, there was a 100 to 200 per cent increase over the normal. After these rats were allowed to "regenerate" their livers, the recoveries again fell to the normal range. On completion of the above, and as a control against the possible influence of operative shock, some of the rats were splenectomized, and in others an abdominal incision was made. Quinine recoveries were again in the normal range.

Except for an expected drop in growth rate immediately following the operative procedures all the rats showed normal growth curves.

This enzymatic conversion of quinine indicates that the effective anti-malarial agent is probably a metabolic product of quinine.

The extraction of proteins from aqueous solution by means of emulsification with chloroform. JAMES C. ANDREWS, W. E. CORNATZER and A. B. SAMPLE. *Dept. of Biological Chemistry, School of Medicine, Univ. of North Carolina, Chapel Hill.* In the removal of proteins from aqueous solution by emulsification with chloroform the following procedure has been adopted: Isoelectric conditions should be employed. The most advantageous proportions are 100 volumes of protein solution, 35 volumes of chloroform and 10 volumes of 95 per cent ethyl or amyl alcohols. This mixture should be shaken vigorously for at least five minutes, then centrifuged and the aqueous layer decanted. Under these conditions, percentage removal (measured by Kjeldahl N.) of albumin solutions ranged from 80 to 98 per cent.

Successive treatments of the aqueous solution as described above caused no significant increase in the percentage of protein removed.

Similar tests of a number of organic liquids of limited water solubility brought to light none superior to the above mixture. The nearest approach was ethyl acetate. Homologs of this ester produced much lower percentage removals.

The precipitated protein is denatured by the process to the point of being insoluble in water, salt solutions, and dilute acid or alkali.

When chloroform alone (without alcohol) was used, the curve of percentage removal *versus* pH reached a low maximum at or near the isoelectric point of the protein. With the alcohol added, this maximum became sharp and much higher.

In the solutions investigated, the protein fraction of higher molecular weight was preferentially precipitated, leaving in solution a fraction with a much higher ratio of free amino to total nitrogen than in the original solution. The lower this ratio was originally, the more effectively was the protein removed.

Para-aminobenzoic acid and hormones. S. ANSBACHER, W. A. WISANSKY and GUSTAV J. MARTIN. *Research Laboratory American Home Products Corp. (IVC Division) Richmond Hill, N. Y. and the Warner Inst. for Therapeutic Research, New York City.* Para-aminobenzoic acid administered orally in daily doses varying from 300 to 600 mg. was observed to be beneficial clinically. In many individuals it seemed to stimulate appetite, to increase libido, and to improve certain asthmatic conditions. It is probable, therefore, that this aromatic amine has a protecting or sparing action on certain hormones.

Data obtained by the Warburg technique showed that p-aminobenzoic acid inhibits the destruction of adrenaline by the enzyme tyrosinase. This observation was confirmed in blood pressure studies on dogs and cats and in dopa-kidney tissue systems. The enzymatic oxidation of stilbestrol was likewise found to be inhibited or retarded by p-aminobenzoic acid as determined by the Warburg technique and by the biological assay with ovariectomised rats. Other hormones are being investigated. The clinical significance of the data will be discussed.

A method of distinguishing endogenous and exogenous fecal phosphorus and the effect of vitamin D on phosphorus absorption. W. D. ARMSTRONG and C. P. BARNUM. *Division of Physiological Chemistry, Univ. of Minnesota, Minneapolis.* Hevesy has shown that several days after the parenteral administration of radiophosphorus, the specific activity (per cent of administered radiophosphorus per mgm. P) of the plasma inorganic phosphorus and urine inorganic phosphorus became equal. The specific activity of fecal phosphorus is lower than that of urine phosphorus indicating that the radioactive endogenous fecal phosphorus is diluted with inactive phosphorus from the food. Since the endogenous phosphorus entering the intestine would also have the same specific activity as that of the plasma and urine,

it becomes possible to calculate, from the specific activity of the feces and the urine phosphorus, the relative amounts of endogenous phosphorus and unabsorbed food phosphorus in feces.

These principles have been made the basis of a long experiment (116 days) in which normal and rachitic rats, on diets of varied calcium and phosphorus content, were employed to test the effect of vitamin D on the mechanism of intestinal absorption. Data will be presented to show that vitamin D had no effect on phosphorus absorption under any condition of calcium or phosphorus content of the diet, but there was some indication that vitamin D facilitated calcium absorption when a low calcium-high phosphorus diet was fed. The animals were given a single injection of 30 x 10^6 counts of radiophosphorus and during the experimental period were fed by stomach tube.

The action of brominated fatty acids on liver fat. CAMILLO ARTOM and MARJORIE SWANSON. *Dept. of Biochemistry, Bowman Gray School of Medicine, Wake Forest College, Winston-Salem, N. C.* It has been shown previously that animals fed iodized natural fats (C. Artom and G. Peretti, Arch. Intern. Physiol. **36**: 351, 1933) or brominated ethyl oleate (C. Zummo, Arch Sci. Biol. **24**; 162, 1938) develop fatty livers. We have now determined the degree of fat infiltration after feeding ethyl esters of fatty acids in which bromine has been substituted on various positions of the carbon chain.

Rats were fed a single massive dose of the brominated ester and the total fatty acids were evaluated in the liver at intervals between 12 find 42 hours after feeding.

The 9, 10-dibromooctadecanoic ester (prepared by bromination of the oleic ester) caused considerable increases in liver fat, amounting to 225 to 375 per cent above normal. Moderate increases (33 to 125 per cent) were obtained after feeding the 13, 14-dibromodocosanoic ester (by bromination of the erucic ester). The slightest increases (8 to 73 per cent) were found after the administration of 6, 7-dibromooctadecanoic ester (by bromination of the petroselinic ester). The 10,11-dibromoundecanoic ester (by bromination of the undecylenic ester) was highly toxic.

The possible significance of these results in relation to the current theories of the metabolism of natural fatty acids will be discussed.

Inhibition of carotene oxidase. A. K. BALLS, B. AXELROD and MARIAN W. KIES. *Bureau of Agricultural Chemistry and Engineering, U. S. Dept. of Agriculture, Washington, D. C.* An enzyme that oxidizes carotene in the presence of unsaturated fats (Sumner and Dounce. Biol. Chem. **134**: 531) is known to exist in many plant tissues and to form products devoid of vitamin A effect (Frey, Schultz and Light. Ind. Eng. Chem. **28**: 1254).

We developed a simple method for measuring the rate of carotene disappearance catalyzed by extracts of soybeans and observed that under the conditions of our experiments (in phosphate buffer pH 6.5) the process is not materially influenced by cystine, glutathione, or cyanide. Traces of chymopapain inhibit, of hydroquinone or pyrogallol accelerate the action. Very small amounts of a protein recently isolated from flour as a crystalline hydrochloride (Balls, Hale and Harris, Cereal Chemistry, March, 1942) protect the carotene from oxidation when added thereto, but not when added first to the soybean extract or to the complete system while oxidation is proceeding. When more fat is used with the carotene, the protection afforded by the protein is less. This is suggestive in view of independent evidence that the protein is a fragment of a lipoprotein in flour. Flour also inhibits carotene oxidation and, like the crystalline protein loses this property on treatment with soybean extract or crystalline chymotrypsin.

The observations seem to indicate a connection, still obscure, between the disappearance of carotene in plant tissues and an inhibitor that acts on the necessary fat, but is destroyed by proteolysis. [*This work was done under Special Research funds provided by the Bankhead-Jones Act.*]

Relation of protein and purine metabolism in pigeons and rats. FREDERICK W. BARNES and the late RUDOLF SCHOENHEIMER. *Dept. of Biochemistry, Columbia University, New York City.* Nitrogen of ammonium citrate, labeled with N^{15} and orally administered to rats and pigeons, is rapidly incorporated in purines of nucleoproteins of internal organs. In the case of the pigeon, 3 per cent of the aspartic acid nitrogen and 6 per cent of the purine nitrogen were derived from the administered ammonia, in a 3-day experiment; the guanine and adenine had practically identical isotope concentrations. As would be expected, there was a high N^{15} level in the excreted uric acid, 15 per cent of the nitrogen of which had been derived from the dietary ammonia.

As the ingested nitrogen also becomes part of the general metabolic pool of nitrogen in the body and is constantly replacing and being replaced by nitrogen of protein origin, it follows that protein nitrogen is convertible to purine nitrogen in both species. Thus the two metabolic paths of purine and protein metabolism are intimately bound together. Uric acid formation in the bird may thus be a special adaptation of a general metabolic process.

Capture, concentration, quantitative determination and elimination of trace metals with tert. butyl-o-nitrosophenol (butylnitroso). OSKAR BAUDISCH and GEORGE HEGGEN. *Research Inst. of The Saratoga Spa, Saratoga Springs, N. Y.* O-nitrosophenol was first synthesized and introduced into chemical analysis by Baudisch (Ber. **45**: 1164, 1912)

Cronheim and Wink developed, in this laboratory, quantitative colorimetric methods for the determination of traces of iron and cobalt (in print). The deep reddish brown, stable cobalt complex formed is easily soluble in petroleum ether. In order to make nitrosophenol compounds especially adaptable to biochemistry, lipoid solubility of many more trace metals would be of paramount importance. Butylnitroso solves this problem. Fe, Cu, Mn, Co, Ni, Pd, Hg, Zn, and other metallic ions can be selectively extracted by using different organic solvents containing butylnitroso. The solubility of the stable butylnitroso metal complexes in different organic solvents is specific. These metals can also be concentrated from aqueous solutions, even if present in infinitesimal amounts, and quantitative colorimetric determinations made. The reagent should be very useful in removing traces from experimental material in nutritional experiments.

Butylnitroso is easily and cheaply prepared by using the Baudisch reaction (Naturwiss. **27**: 768, 1939; Science **2389**: 336, 1940; J. Am. Chem. Soc. **63**: 622, 1941). The method is described in detail. The quantitative colorimetric procedure for the determination of extremely small amounts of iron is given. Concentrations of the green iron complex in ligroin as low as 0.2 gamma/cc. give accurate determinations. Iron was concentrated and determined in water solutions at dilutions of 3 gamma/1000 cc. (1:300,000,000).

The utilization of amino acids for growth in the mouse. CLIFFORD D. BAUER and CLARENCE P. BERG *Biochemical Laby., State Univ. of Iowa, Iowa City.* Mice fed a cystine-deficient casein hydrolysate supplemented with tryptophane grew less rapidly than mice fed the same diet plus cystine. Contrary to the findings of others, methionine and cystine proved equally effective.

Moderate but rather varied growth was obtained on a diet containing a mixture of 20 purified amino acids (Rose and Rice. J. Biol. Chem. **130**: 305, 1939) at an active level of 20.5 per cent as the source of protein nitrogen. Omission of any of the 10 amino acids essential for the rat, except arginine, induced loss in weight. Whether arginine can be synthesized rapidly enough for maximum growth is uncertain. When only the 10 amino acids were supplied at a 20.5 per cent active level, growth occurred at a diminished rate.

Both optical isomers of methionine and phenylalanine were effective in promoting growth, but only the natural isomers of valine, leucine, isoleucine, and threonine could be utilized. Isomers of arginine, lysine, and tryptophane have been tested previously.

Threonine deficiency led to an onset of edema and an accumulation of ascitic fluid. When the vitamin B complex was supplied as a mixture of ryzamin-B, thiamin chloride, riboflavin, and nicotinic acid, d(+)-histidine checked loss in weight somewhat but did not promote growth. On similar diets rats grew well. Yeast vitamin Harris concentrate allowed essentially the same growth on d(+)-histidine as previously reported. No satisfactory explanation for this discrepancy has been found.

Induced hypoprothrombinemia and vitamin C excretion. C. A. BAUMANN, J. B. FIELD, R. S. OVERMAN and K. P. LINK. *Dept. of Biochemistry, College of Agriculture, Univ. of Wisconsin, Madison.* The ingestion of the anticoagulant 3,3′-methylenebis(4-hydroxycoumarin) induced a hypoprothrombinemia in rats fed diets moderately low in vitamin K. The hypoprothrombinemia could be reduced markedly by the administration of substances such as carvone, chloretone, aminopyrine, and paraldehyde, all of which stimulate the excretion of reducing substances by the rat. The ability of any of these substances to nullify the anticoagulant appeared to be roughly proportional to its effectiveness in promoting the excretion of vitamin C. The various substances did not affect the prothrombin time of plasma from rats which received no anticoagulant. The responses were uniform when the basal diet consisted of condensed milk, but variable responses resulted when a semisynthetic basal diet was fed.

Vitamin C itself did not reduce the hypoprothrombinemia induced by the anticoagulant, nor did the vitamin prolong the lives of animals receiving the anticoagulant daily. However, the lives of such animals were prolonged by compounds which stimulated the excretion of vitamin C.

A nutritional assay of oxycasein by growth experiments with albino rats. MARY ADELIA BENNETT and GERRIT TOENNIES. *Lankenau Hospital Research Inst., Philadelphia.* The basal diets used consisted of protein, 0.4 per cent l-cystine, dextrin, 15 per cent sucrose, 4 per cent salt mixture, 2 per cent agar and crystalline fat soluble vitamins in 30 per cent corn oil. Eight pure B-vitamins were given daily in sucrose solution by mouth. In experiment I choline chloride (20 mgm.) and amino acid supplements were fed separately in butter; in experiment II choline chloride (0.40 per cent) and amino acids were incorporated in the diet.

In experiment I (seven male litter mates) the basal protein was 6 per cent oxycasein (Toennies, these Proceedings). Oxycasein alone and oxycasein supplemented with dl-methionine (15 mgm.) plus dl-threonine (79 mgm.) or l-tryptophane (13 mgm.) plus threonine produced similar, steadily declining weight curves, while supplementation of oxycasein with methionine plus tryptophane yielded a satisfactory growth rate, unimproved by addition of threonine.

In experiment II the growth response (six animals each) to *a*, 10 per cent casein supplemented with 0.10 per cent tryptophane was compared with that to *b*, 10 per cent oxycasein supplemented with 0.20 per cent tryptophane and 0.34 per cent methionine. Control groups (three animals each) receiving oxycasein *c*, oxycasein plus methionine *d*, and oxycasein plus tryptophane *e*, showed, during 12 days, losses of 13.3 ± 1.1 (av. dev.), 13.5 ± 0.8 and 15.5 ± 0.5 per cent respectively, while group (a) gained 47 ± 7 and group (b) 31 ± 5 per cent. Average daily food consumption (grams per rat) was *a*, 6.4; *b*, 5.1; *c*, 2.1; *d*, 2.0, and *e*, 1.9.

The determination of tyrosine and related phenols in blood filtrates by means of the Millon reaction. F. W. BERNHART and D. ROY MCCULLAGH. *Dept. of Biochemistry, Cleveland Clinic Foundation, Cleveland, O.* The Millon reaction may be employed for the determination of tyrosine and related phenols in blood filtrate by using the photoelectric colorimeter. 1:5 filtrate is prepared by adding 2 volumes of water, 1 volume of 10 per cent sodium tungstate and 1 volume of 1N sulfuric acid to 1 volume of oxalated blood. To 7 cc. of filtrate in a test tube calibrated at 10 cc. are added 0.5 cc. of 7N sulfuric acid, 1.5 cc. of 15 per cent mercuric sulfate in 6N sulfuric acid and 1 cc. of water. To the standard tube containing 7 cc. of the same filtrate and the same reagents is added 1 cc. of tyrosine solution (0.05 mgm. in 1 cc.). The tubes are heated 8 minutes in a boiling water bath, then cooled below room temperature in running water. After adjusting the volume to 10 cc., the tubes are centrifuged, and 8 cc. of the clear liquid are placed in the colorimeter cell. The blank reading is determined, 0.05 cc. of 5 per cent sodium nitrite solution is added, and the mixture is shaken. The color produced is read after 4 minutes using a filter with maximum transmission at 500 millimicrons. Tyrosine (4 mgm.) added to 6 cc. of blood is quantitatively recovered by this method. Spectrophotometric analysis shows that color produced in blood filtrate has the same absorption maximum as the color produced in tyrosine solution.

By this method the phenol concentration of 100 cc. of whole blood is equivalent to 1 to 2 mgm. of tyrosine.

The oxidation of benzoate and related compounds by certain mycobacteria. FREDERICK BERNHEIM. *Dept. of Physiology and Pharmacology, Duke Univ. School of Medicine, Durham, N. C.* The tubercle bacillus can oxidize benzoic acid and o-hydroxybenzoic acid but not m- or p-hydroxybenzoic acids. A non-pathogenic mycobacterium, an unnamed species obtained from Dr. Van Niel's collection, oxidizes benzoic and m- and p-hydroxybenzoic acids but not o-hydroxybenzoic acid. The amino benzoic acids are not oxidized by either. Unlike the tubercle bacillus, the non-pathogenic species oxidizes phenol and tyrosine. Phenylalanine and other amino acids are not attacked. Like the tubercle bacillus the non-pathogenic species oxidizes aldehydes and fatty acids from acetic to palmitic. Benzoic acid is oxidized by the non-pathogenic species with an uptake of 8 atoms of O_2 and a production of 4 molecules of CO_2 per molecule. The corresponding values for m- or p-hydroxybenzoic acids are 7 atoms of O_2 and 4 molecules of CO_2; for tyrosine, 8 atoms of O_2, 3 molecules of CO_2 and ammonia; for phenol, 10 atoms of O_2 and 4 molecules of CO_2. The oxidations occur more rapidly at pH 7.8 than at pH 6.7. Various iodine substituted benzoic acids inhibit the oxidation of the ring compounds.

Influence of nephrectomy on ovarian response to gonadotropins. FRITZ BISCHOFF and GEORGENA J. CLARKE. *Chemical Laby., Santa Barbara Cottage Hospital Research Inst., Santa Barbara, Calif.* Twenty-two day-old rats were partially nephrectomized by the technic of Chanutin and Ferris. Four days later body weight was approximately 20 per cent below that of litter-mate controls, heart weight was equal to that of controls, the blood non-protein nitrogen was elevated 30 mgm. per 100 cc., and the blood pressure was slightly elevated. Solutions of sheep pituitary gonadotropin, prolan, or purified pregnant mare serum hormone were administered to operated and to intact litter-mate rats. The influence of partial nephrectomy follows:

The augmented response to pituitary hormone was of the same order as that produced by thyroidectomy, and much less than that produced in intact rats by divided dosage or administration of an insoluble compound. The augmented response to mare serum hormone could be accounted for by body weight difference (same as the influence of thyroidectomy). The augmented response to prolan, which was 175 to 240 per cent, was greater than that produced by divided dosage, thyroidectomy, or administration of an insoluble form. Prolan was able to produce ovaries weighing more than 100 mgm. at the age of 26 days in partially nephrectomized rats.

The well known failure of prolan, in contrast to sheep pituitary and mare serum hormones, to produce increases in ovarian weight of intact rats proportional to increases in level of hormone administered is interpreted as due to a low renal threshold for prolan.

The fecal and urinary excretion, the tissue distribution, and the serum concentration of bromide and chloride during prolonged bromide administration. OSCAR BODANSKY and WALTER MODELL. *Dept. of Pharmacology, Cornell Univ. Medical College, New York City.* Dogs were placed on diets containing known amounts and constant

ratios of bromide to chloride for periods varying from about three weeks to two and a half months. For the different animals, the ratio of bromide to total halide ingested (Br_i/H_i) ranged from 0.29 to 0.57.

The concentration of serum halide remained constant, within slight variation, during the period of administration. The concentration of serum bromide rose until it approached or eached an equilibrium value at which the ratio of the percentage of bromide in the serum halide to the percentage in the food halide, i.e., $\frac{Br_s/H_s}{Br_i/H_i}$ was a constant, K', equal to 1.43 (a.d. = 0.05). The rate of rise of serum bromide depended both upon the values of Br_i and Br_i/H_i.

As bromide accumulation progressed, the ratio of the percentage of bromide in the urinary halide to the percentage in the blood serum, i.e., $\frac{Br_u\ H_u}{Br_s\ H_s}$ increased; in other words, the differential excretion of bromide and chloride decreased. Fecal excretion of bromide also became appreciable, amounting in some metabolic periods to 20 per cent or more of the total bromide excreted. The occurrence of fecal excretion of bromide in more than negligible quantities has not hitherto been reported and should be taken into account in calculating extracellular body fluid by the bromide method. The amount of bromide retained (bromide ingested minus bromide excreted in urine and feces) was often greater in later stages of accumulation than could be accounted for by assuming distribution in the normal volume of extracellular fluid.

The effect of feeding tryptophane upon the urinary output of acetone bodies. RAYMOND BORCHERS and CLARENCE P. BERG. *Biochemical Laby., State Univ. of Iowa, Iowa City.* Tryptophane, indole derivatives, and kynurenine form precipitates with mercuric sulfate. For the determination of total acetone bodies by Van Slyke's procedure, urines voided by rats fed large doses of tryptophane were acidified to precipitate kynurenic acid; the clarified filtrate was strongly acidified with sulfuric acid, and preformed acetone and the acetone formed by the decomposition of the acetoacetic acid were distilled and collected in a sulfuric acid solution of mercuric sulfate; mercuric sulfate was added to the residue to precipitate the interfering compounds; the filtrate was added to the acetone distillate, and total acetone bodies were determined on this mixture.

In fasting female rats, 0.6 gram of *l*-tryptophane fed daily for 4 days in suspension in gum tragacanth increased the average output of acetone bodies slightly over that of controls fed the tragacanth solution alone. When sodium butyrate was fed simultaneously, the average acetone body output in the animals fed tryptophane was markedly lowered.

dl-Tryptophane produced essentially the same acetone body output, but much less kynurenic acid; the mercuric sulfate precipitate containing the interfering metabolites was usually larger.

The studies suggest that tryptophane is ketolytic (or antiketogenic) when fed to the animal excreting large amounts of acetone bodies, but may either produce acetone bodies or facilitate their excretion in animals eliminating only small amounts. Similar tests are being conducted on *d*-tryptophane and on tryptophane metabolites.

Chemical differentiation between the chorionic gonadotropic hormone and serum proteins in aqueous solution. DONALD E. BOWMAN. *Dept. of Biochemistry and Pharmacology, India Univ. School of Medicine, Indianapolis.* The use of a qualitative test for the chorionic gonadotropic hormone, employing phenylhydrazine, has been described and necessary precautions have been given by several investigators. (C. Dolf. Zentralbl. f. Gynäk. **59**: 2901, 1935; E. Wiesner. Zentralbl. f. Gynäk. **60**: 1588, 1936; B. Friedrich. Monatschr. f. Geburtsh. u. Gynäk. **103**: 211, 1936; K. Patkay. Arch. Gynäkol. **169**: 13, 1939). Outstanding among the precautions is the necessity of avoiding small amounts of serum proteins. This is of considerable practical disadvantage. The object of the present report is to describe a procedure, using the same reagent, which can be employed to differentiate between small amounts of the hormone and serum proteins in aqueous solution.

With the hydrogen ion concentration controlled by phosphate buffer, the chorionic gonadotropic hormone produces a marked, fine, permanently suspended, colloidal precipitate when heated for a short time in the presence of definite proportions of phenylhydrazine hydrochloride, sodium acetate, and hydrogen peroxide. The density of the precipitate is a function of the concentration of the hormone which can be estimated nephlometrically. Serum proteins produce the usual type of coagulum. In a preliminary step the coagulable proteins can be removed by heating in the presence of KH_2PO_2.

As a source of material the hormone prepared according to Gurin, Bachman, and Wilson and most of the commercially available preparations were employed. For quantitative estimation, a concentration equivalent to 5 to 25 rat units is most suitable. Glucose, lactose and the usual urinary constituents give results essentially the same as a blank.

Colorimetric determination of serine. M. JOHN BOYD and MILAN A. LOGAN. *Dept. of Biological Chemistry, Univ. of Cincinnati Medical College, Cincinnati, O.* A colorimetric method which is suit-

able for the determination of small amounts of serine (1 to 5 mgm.) has been developed. Formaldehyde, which is formed by the action of periodic acid on serine, is quantitatively distilled from the reaction mixture without decomposition of periodic acid. The formaldehyde thus obtained is estimated colorimetrically with the aid of 1,8-dihydroxynaphthalene - 3,6 - disulfonic acid, the error not exceeding 1 to 2 per cent. The reagent produces no color with acetaldehyde or higher homologues. Fifty to 100 micrograms of formaldehyde in a volume of 50 cc. produces adequate color for determination in a Duboscq colorimeter. Color production is proportional to the concentration of formaldehyde (within 1.5 per cent) when the unknown differs from the standard by not more than 50 per cent.

Carbohydrates which are thought to be present in proteins do not cause erroneous results in serine determinations.

The purity of various cysteine preparations has been investigated. The serine content of purified casein, collagen, crystalline hemoglobin, salmin, and egg albumin has been determined. The stability of serine during acid hydrolysis is discussed.

Minimum molecular weight (M_{min}) of β-lactoglobulin. ERWIN BRAND and BEATRICE KASSELL. *Dept. of Biochemistry, Columbia Univ., New York City.* A sample of recrystallized native β-lactoglobulin (obtained from Dr. R. Keith Cannan) was analyzed for total ND (15.60 per cent), methionine (3.22 per cent; 0.692 per cent S), cysteine + cystine (3.39 per cent, 0.905 per cent S), cysteine (1.10 per cent), cystine (2.29 per cent), total S (Pregl 1.60 per cent; methionine + cysteine + cystine S = 1.60 per cent), tyrosine (3.78 per cent), tryptophane (1.94 per cent) (for methods, cf. analysis of chymotrypsinogen[1]).

Since lactoglobulin is known to be homogeneous, its constituents must be present in integral numbers. The smallest whole number ratios for 3 constituents are calculated below, indicating methionine:1 (cysteine + half-cystine) = 3:4, and tryptophane:methionine = 4:9.

$$\frac{\text{per cent methionine S}}{\text{per cent (cysteine + cystine) S}} = \frac{0.692}{0.905} = \frac{1}{1.31} = \frac{3}{3.92};$$

$$\frac{\text{per cent tryptophane} \times M_{\text{methionine}}}{\text{per cent methionine} \times M_{\text{tryptophane}}} = \frac{289}{657} = \frac{1}{2.27} = \frac{4}{9.08}.$$

From these ratios and the percentages found, M_{min} is calculated (J. Gen. Physiol. **25**: 167) to be 42,000, identical with M_s (42,000 at pH 5.4 and 6.1) obtained by Pedersen (Biochem. J. **30**: 961) from sedimentation velocity and diffusion. A molecule of lactoglobulin contains 9 methionine, 4 cysteine, 8 half-cystine (4 S-S groups), 9 tyrosine, 4 tryptophane residues, and a total of 468 nitrogen atoms.

Doctor Cannan has kindly communicated to us values for amide and basic amino acid content; from these and the tryptophane the number of non-α-amino N atoms is estimated as approximately 100. A molecule of lactoglobulin, therefore, contains approximately 368 amino acids with an average residue weight of 114.

Metabolism of N-derivatives of amino acids. FRED G. BRAZDA and JAMES C. RICE. *Depts. of Biochemistry and Pharmacology and Experimental Therapeutics, School of Medicine, Louisiana State Univ., New Orleans.* In this study the metabolism of certain N-derivatives of amino acids was investigated to ascertain whether these compounds may be oxidatively deaminated without previous hydrolysis. Such deamination without hydrolysis could have relation to polypeptide degradation and possibly even to the mechanism of urea formation. The study of hydantoins and their derivatives is obviously related to the clarification of the mode of action of these compounds as drugs.

N-acetyl-dl-phenylalanine caused a marked increase in utilization of oxygen by rat liver slices. dl-methylhydantoic acid caused a less marked increase in oxygen utilization. Neither compound caused an increase in oxygen uptake of rat kidney slices. dl-5-methylhydantoin did not cause extra oxygen utilization by kidney or liver slices. None of the compounds mentioned was catalytically deaminated by d-amino acid dehydrogenase.

Chemical studies to determine the course of intermediate reactions are in progress.

Carbohydrate formation after feeding l(—) histidine. JOSEPH S. BUTTS and LE MAR F. REMMERT. *Biochemical Laby., Chemistry Dept., Oregon State College, Corvallis.* In a continuation of our studies of amino acid metabolism in relation to conversion to carbohydrate, this report deals with the role of l(—) histidine in such a transformation.

Female rats were fed a high fat, low protein diet for eight days. At the end of this period the animals were placed in individual cages and after a preliminary fast period were fed by stomach tube an aqueous solution of l(—) histidine, adjusted to a pH of 6.5. One group of rats received 8.5 grams of the acid per square meter of body surface, while a second group was fed twice this amount. Urine collections were made daily, and the urine was analyzed for acetone bodies and nitrogen.

In all experiments, after what appeared to be a latent period, the ketonuria was markedly low-

ered, with the larger dose being more effective than the smaller.

The urinary excretion of histidine was found to be practically nil, even after feeding as much as one gram of the acid.

In a second phase of the study, liver glycogen determinations were made after feeding to rats, which had been previously starved forty-eight hours, an average dosage of 400 mgs. of l(—) histidine. The animals were sacrificed at 4 hour intervals. At the end of the 4 and 8 hour periods, no glycogen formation occurred but at twelve hours some deposition was found with a maximum amount at sixteen hours (1.08 per cent). At 20 hours the level had begun to drop.

Some observations on the α-keto acid derived from methionine. WILLIAM M. CAHILL and GUILFORD G. RUDOLPH. *Dept. of Physiological Chemistry, Wayne Univ. College of Medicine, Detroit.* There is decisive evidence for the belief that methionine may be oxidatively deaminated in the animal organism. It has remained, nevertheless, for a study to be made on the replaceability of this amino acid in the diet of rats with its keto acid analogue. It was found possible to prepare the keto acid for this purpose by splitting the corresponding 2,4-dinitrophenylhydrazine derivative by heating with an aqueous solution of acetone in a pressure bottle, and subsequently removing the acetone and its substituted hydrazone. The 2,4-dinitrophenylhydrazone for this treatment was prepared in quantity by taking advantage of an interesting reaction observed some years ago by Bergmann and Stern (Ann. Chem. **448**: 30, 1926) when working with alanine. In our case, α-bromopropionylmethionine was allowed to stand for 10 minutes with acetic anhydride and anhydrous sodium acetate, the mixture was heated for 5 minutes in the water bath with dilute hydrochloric acid, and the desired keto acid which was formed as one of the products of the decomposition was precipitated from the cooled reaction mixture as the 2,4-dinitrophenylhydrazone. The sodium salt of α-keto-γ-methylmercaptobutyric acid was found to be capable of replacing *dl*-methionine for growth in young white rats fed a diet deficient in this amino acid. The compound was effective whether incorporated in the diet or injected subcutaneously.

The antioxygenic action of ascorbic acid with quinone. VINCENT P. CALKINS and H. A. MATTILL. *Biochemical Laby., State Univ. of Iowa, Iowa City.* To determine why ascorbic acid acts as a stabilizer of fat against oxidative rancidity in the presence of inhibitols, a kinetic study of the system lard ethyl esters-quinone-ascorbic acid was made. Velocity constants were determined for the oxidation of ascorbic acid in lard ethyl esters in the presence and absence of quinone. From these velocity constants, by the theory of absolute reaction rates, the free energy of formation of the activated state of each system was determined. This showed that quinone had lowered the energy of activation, by serving as an intermediate catalyst. The presence of quinone permitted ascorbic acid to react in its normal monovalent fashion, with fat peroxides, thus avoiding the high energy of activation necessary for it to react in bivalent fashion. Quinone was reduced to hydroquinone which could react bivalently with the fat peroxides.

The large synergistic effect obtained was not due to the primary oxidation of ascorbic acid, for this had completely disappeared in a few hours; the effect was due mainly to the further oxidation products of ascorbic acid, namely dehydroascorbic acid, threonic and oxalic acids. By a newly developed microcolorimetric method for the determination of oxalic acid, it was found that the trace of hydroquinone present was maintained constant by the oxidation of oxalic acid, which latter substance was likewise maintained constant, presumably through constant oxidation of threonic acid.

The effect of parathyroid extract upon the distribution and excretion of labeled phosphorus. W. WESLEY CAMPBELL and WILBUR R. TWEEDY. *Dept. of Biological Chemistry, Loyola Univ. School of Medicine, Chicago, Ill.* One hundred (Collip) units of parathyroid extract (Lilly) were administered subcutaneously to each of 8 young adult rats. One hour later a solution of 7.5 mgm. of Na_2HPO_4, containing 5 to 10 microcuries of radiophosphorus, was administered intraperitoneally or by stomach tube to these animals and to an equal number of controls. The animals were sacrificed at 4 hours, 24 hours, and 8 days.

During the first 24 hours larger amounts of labeled phosphorus were found in the livers, kidneys, and urine of the hormone treated animals than in their controls. In the animals injected intraperitoneally, less radiophosphorus appeared in the feces of those treated with parathyroid extract.

After 24 hours the rate and amount of labeled phosphorus excreted in the feces was similar in the hormone treated and control animals. The same observation was made with respect to the urinary excretion.

The deposition of the labeled phosphorus in the femurs of the control animals exceeded that in the femurs of the hormone treated animals. [*We are greatly indebted to Prof. E. O. Lawrence, director, and to Dr. Joseph G. Hamilton and the rest of the staff of the Radiation Laboratory of the University of California for supplying the radiophosphorus used in these experiments. This work was also aided in part by Grant 535 from the*

American Medical Association, and a gift of parathyroid extract from Eli Lilly and Company.

The distribution of water and electrolytes between blood, fluids and skeletal muscle in pregnant dogs. ALICE CHILDS and LILLIAN EICHELBERGER. *Dept. of Medicine and the Lasker Foundation for Medical Research, Univ. of Chicago, Ill.* Data are given on 1, the distribution of water and electrolytes between blood and skeletal muscle in the pregnant organism, and 2, on the changes in water and electrolyte distribution between blood, fluids and skeletal muscle following increases in total body water produced by the intravenous injection of large volumes of an isotonic solution containing 25mM $NaHCO_3$ + 129 mM NaCl. These data are compared statistically with those obtained previously on non-pregnant dogs. The dogs studied were in the late stages of pregnancy.

Muscles from the pregnant dogs did not differ in water distribution or electrolyte content from the muscles of non-pregnant animals. Following the increases in body water the additional water in skeletal muscle was distributed as in normal muscle. Therefore in these experiments there was no indication of any influence of pregnancy on the distribution of fluid in skeletal muscle.

In order to examine further the distribution of the increased total body water in the pregnant organism, the relative concentrations of water and electrolytes in the urine and in the peritoneal, amniotic and allantoic fluids are discussed.

Isolation of a purified protein with marked thyrotropic activity from beef pituitary tissue. LEON S. CIERESZKO and ABRAHAM WHITE. *Dept. of Physiological Chemistry, Yale Univ. School of Medicine, New Haven, Conn.* A purified protein with marked thyrotropic activity has been prepared from beef pituitaries. The product is active at a total dose of one microgram, as judged by thyroid histological response in the chick. The behavior of this protein in the Tiselius apparatus and in the ultracentrifuge indicates that it is a homogeneous product of relatively low molecular weight.

Frozen, whole beef pituitaries are ground and stirred with 2 per cent NaCl solution (5 liters/kgm. tissue) for 3 hours at pH 7.8 and 0° to 5°C. The centrifuged supernatant is adjusted to pH 4.1, centrifuged, and an equal volume of acetone added to the supernatant. After standing overnight in the ice-box, the precipitate is centrifuged. The supernatant is again treated with an equal volume of acetone; after several hours the precipitate is centrifuged and washed thoroughly with 80 per cent acetone. It is then extracted with several small portions of water and the combined extracts adjusted to pH 9.0. The precipitate is centrifuged, the supernatant adjusted to pH 7.0, and 5 per cent lead acetate added until no further precipitation occurs. The precipitate is separated and 1.25 M trichloroacetic acid added to the supernatant solution to a final concentration of 0.5 M. A precipitate slowly flocculates; this is centrifuged. The clear, colorless supernatant is dialyzed, concentrated by pervaporation, and lyophilized. The product is a white solid; it is very soluble in water, gives the usual protein tests and a positive Molisch reaction.

The distribution of vitamin A, carotene and xanthophyll in the tissues of man and animals. S. W. CLAUSEN and A. B. MCCOORD. *Dept. of Pediatrics, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.* The concentrations and contents of vitamin A and carotenoid pigments in the liver, heart, lungs, spleen, pancreas, testes or ovaries, kidneys, thyroids, adrenals, eyes, brain, subcutaneous fat, retroperitoneal fat, muscle, skin, skeleton, gastro-intestinal tract, blood and urine of man, monkey, dog, cat, rat, guinea pig, rabbit, gopher and chicken were determined. Vitamin A was found in almost all tissues examined. While there was considerable variation among the species, vitamin A was stored mainly in the liver, and in the ester form. A small amount of the free form was always present in the tissues. A considerable amount of vitamin A was also stored in the fat depots, particularly of the dog and cat. The concentration of the vitamin was high in the kidneys and adrenals of many subjects. Almost all of the vitamin A in the blood, with the exception of that of the dog and cat, was in the free form. We have found the vitamin in the urine of the normal monkey and dog, and in that of children suffering from chronic nephritis.

Carotene and xanthophyll in varying concentrations were usually found in the livers of all subjects, but other tissues of the dog, rabbit, rat and gopher did not contain significant amounts of the pigments. The pigments were present in the fat depots and adrenals of man, monkey, guinea pig, cat and chicken. Carotene and xanthophyll were found in the blood and in many other tissues of man, monkey and chicken.

The molecular size and shape of the nucleic acid of tobacco mosaic virus. SEYMOUR S. COHEN (Fellow in the medical Sciences of the National Research Council) and W. M. STANLEY. *Dept. of Animal and Plant Pathology, Rockefeller Inst. for Medical Research, Princeton, N. J.* Nucleic acid of the ribose type may be obtained in protein-free solution by heat denaturation of tobacco mosaic virus nucleoprotein. Examination of this solution and its concentrates by means of diffusion, rate of sedimentation, electron microscopy and osmotic pressure revealed a single component, the particles of which were highly asymmetric and had a molecular weight of approximately 300,000. This material on standing in 0.1 M $(NH_4)_2SO_4$ for several weeks decomposed to form a less asym-

metric nucleic acid having a molecular weight approximately one-sixth that of the original material and apparently homogeneous with respect to diffusion and sedimentation. A homogeneous sample of nucleic acid of similar size has also been isolated directly from heat-denatured tobacco mosaic virus.

A sample of nucleic acid obtained by Dr. H. S. Loring after alkaline denaturation of the virus was found to consist of a mixture of substances. Dialysis removed low molecular weight material, amounting to approximately 50 per cent of the total phosphorus present. Examination of the dialyzed solution by means of electrophoresis revealed a single component having the same electrophoretic mobility as that of yeast nucleic acid and of the material isolated after heat denaturation of the virus. The molecular weight of this component, as derived from sedimentation and diffusion measurements, was approximately 11,000. The significance of these results with respect to the structure of ribose nucleic acid and tobacco mosaic virus will be discussed.

The biochemistry of mineral deficiency. III. Mineral balances. ELMA TUFTS COHN and DAVID M. GREENBERG. *Division of Biochemistry, Univ. of California Medical School, Berkeley.* Magnesium deficiency, when the calcium and phosphorus content of the diet is normal, was found to be characterized by the following changes in mineral metabolism:

1. There is a large loss of magnesium divided equally between feces and urine during the first few days of the deficiency, then, a minimal loss with only about one-fifth as much in urine as in feces, and finally, in the last stage, an increasing loss of magnesium, appearing mainly in the feces.

2. There is an increased retention of calcium during the first few days of the deficiency followed by increasing losses of calcium via the feces in the second stage (associated with anorexia), which eventually changes the calcium balance from positive to negative.

3. The phosphorus retention also is increased in the first stage of the deficiency and then decreases in the second stage. The greater part of the loss of phosphorus is via the urine.

Increasing the calcium level in the diet tends to reduce the magnesium retention. Raising both the calcium and phosphorus in the diet lowers the retention still more.

The reduction in magnesium retention appears to be accounted for partly by a loss of magnesium occluded on the insoluble calcium phosphate in the feces and partly by a reduced food intake.

Non-utilization versus overproduction of glucose by the depancreatized dog. JEROME W. CONN, ELIZABETH STERN CONN and MARGARET W. JOHNSTON. *Nutrition Laby., Dept. of Internal Medicine, Univ. of Michigan Medical School, Ann Arbor.* Completely depancreatized dogs were maintained in good health with insulin and a diet containing raw pancreas. Insulin and food were then withheld until constant D/N ratios were obtained. Blood-sugar remained between 250 and 380 mgm. per cent. Under these conditions continuous indirect calorimetry yielded total R.Q.'s of about 0.71. The animals were then phloridzinized. Within 6 to 12 hours the blood sugar fell to hypoglycemic levels. While the blood sugar was falling the D/N ratios rose as high as 16/1 but there was no significant change in the R.Q.'s. When the blood sugar stabilized in the hypoglycemic range, the D/N ratios returned to the pre-phloridzin level, the R.Q.'s always remaining in the region of 0.70.

If at a blood sugar level of 30 mgm. per cent the animals' capacity to utilize carbohydrate had been reduced from that obtaining at 380 mg. per cent, and if conversion of fat to carbohydrate in the liver were continuing (both of these phenomena being postulates of the overproduction hypothesis), two results would have been expected in our experiments: 1, a fall of the total R.Q. far below 0.70, and 2, a rise of the D/N ratio above that observed in the pre-phloridzin period. Also, had the extra glucose excreted during the period of falling blood sugar been derived from fat, its unavailability for oxidation should have resulted in a significant fall of the R.Q. None of these phenomena were observed.

The data are readily explained on the basis of essential non-utilization of carbohydrate by the depancreatized dog.

Poising of plasma. THOMAS B. COOLIDGE. *Dept. of Biochemistry, Duke Univ. School of Medicine, Durham, N. C.* The system poising the oxidation-reduction potential of plasma and urine has been further purified. In the most extensively purified preparations it is associated with a yellow pigment. This pigment is one of the so-called "urochrome" group in confirmation of the work of Barrenscheen, but most of the other urinary pigments are not involved.

The Van den Bergh reaction. THOMAS B. COOLIDGE. *Dept. of Biochemistry, Duke Univ. School of Medicine, Durham, N. C.* By protecting the plasma bilirubin from oxidation by means of ascorbic acid it has been possible to purify it, along with the albumin fraction to which it is attached, by fractional precipitation with ammonium sulfate. The bilirubin is attached to those fractions of the plasma albumin which are precipitated by higher concentrations of ammonium sulfate. The ascorbic acid does not affect the "directness" of the reaction.

Serum protein regeneration as effected by intravenously administered protein hydrolysates.

WARREN M. COX, JR., ARTHUR J. MUELLER and K. S. KEMMERER. *Mead Johnson Labs., Evansville, Ind.* Enzymatic hydrolysates of casein, lactalbumin and beef serum protein were made by pancreatic digestion and the dried hydrolytic products compared with the original protein by feeding to rats at comparable intake levels of 2.4, 1.7 and 1.2 per cent nitrogen. All comparisons indicated that the hydrolysates were as effective in promoting growth as the intact protein.

Unanaesthetized dogs made hypoproteinemic by feeding a low protein diet for three weeks were given seven daily intravenous infusions of a sterile 10 per cent solution of the hydrolysates. Supplementation was at a protein equivalent level of 2.5 grams per kilo body weight. Control animals were fed an equal amount of the hydrolysate. Forty-one periods of supplementation were observed.

Regeneration of serum protein occurred with all three hydrolysates. The casein hydrolysate by mouth gave an average assay value (Weech and Goettsch) of +0.35 per cent. Intravenously, the assay value was +0.43 per cent. Lactalbumin by mouth showed an assay value of +0.32, and by vein an identical figure. Preliminary assay values for serum protein hydrolysate were +0.42 by vein (3 observations) and +0.82 by mouth (2 observations). Thus, enzymic casein and lactalbumin hydrolysates are equally effective in serum protein regeneration in hypoproteinemic dogs. The hydrolysates are as effective intravenously as orally.

Synthesis of amino acids by the chick embryo. FRANK A. CSONKA and HARRY W. TITUS. *Bureau of Agricultural Chemistry and Engineering, and the Bureau of Animal Industry, U. S. Dept. of Agriculture, Washington, D. C.* This investigation was undertaken for the purpose of ascertaining whether or not there is synthesis of amino acids by the developing chick embryo. Accordingly, quantitative determinations were made of certain individual amino acids—cystine, threonine, tyrosine, tryptophane, arginine, histidine, and lysine—in the eggs of chickens on both high- and low-protein diets and in chicks freshly hatched from such eggs. Both high- and low-protein diets were fed to the chickens in order to find out if the level of protein intake influenced the amino acid content of the resulting egg.

The data that have been obtained suggest that the arginine, cystine, and tyrosine contents of the egg are larger when a high-protein diet is fed than when a low-protein diet is fed. In general, there was more of each of the several amino acids, except cystine, in the eggs than in the chicks. The quantity of tyrosine in the chicks was significantly less than in the eggs. The data also suggest the possibility of some cynthesis of cystine by the developing embryo.

Nutritional achromotrichia in rats. F. PEIRCE DANN, RUTH C. MOORE and D. V. FROST. *Abbott Labs., North Chicago, Ill.* Nutritional achromotrichia was studied both curatively and prophylactically. Depigmentation was developed in 28 day rats with an original diet (Proc. Soc. Exper. Biol. and Med. **46**: 507, 1941) supplemented by: 1 g. of cystine, 60 mgm. each of $CuSO_4 \cdot H_2O$ and $ZnSO_4 \cdot 7H_2O$, 20 mgm. of inositol and 300 gammas of d-Ca pantothenate per 100 grams of diet. Depleted rats were continued on the basal diet plus different test materials for 7 more weeks. Of 125 test rats on 100 to 500 gammas of d-CA pantothenate daily, 70 were not cured, 54 were partially cured, and 1 was definitely cured. No rats were cured on 3 mgm. of p-aminobenzoic acid (PAB) while 4 out of 13 were partially cured on PAB plus d-Ca pantothenate. 15 rats of 26 on Ansbacher's diet from the 28th day, became gray and were fed 3 mgm. of PAB for 7 weeks more. 4 were not cured, 11 were partially cured.

Three groups of 24 day old rats, 15 in each, were fed the following diets: 1, original diet including the above supplements plus (daily): 0.037 gammas of biotin (injected), 1 mgm. of ascorbic acid, 3 mgm. of PAB, 200 gammas of d-CA pantothenate; 2, the basal diet plus 10 per cent brewer's yeast; 3, the basal diet plus 20 per cent whole liver powder. Eleven rats in group 1 developed grayness; none in groups 2 and 3 became gray.

Conclusions: 1. PAB had no effect on graying alone or slight if any supplementary effect with d-Ca pantothenate. 2. Addition of all known B factors including PAB did not prevent graying. 3. An unknown factor(s) is again postulated.

Two forms of phosphorylation of glucose in human erythrocytes and their relation to the system cozymase-dihydrocozymase. ZACHARIAS DISCHE. *Labs. of Mount Sinai Hospital, New York City.* Two different enzymatic systems of phosphorylation of glucose are present in intact erythrocytes. One catalyzes the transphosphorylation between adinosinepolyphosphoric acids and glucose. The other is coupled to the oxidoreductive processes involved in glycolysis and is independent of the presence of any measurable amount of adinosinepolyphosphoric acids. Both systems of phosphorylation are active in diluted hemolysates in the presence of glucose, 0.04 M NaF and Na pyruvate. Only a part, however, of the formed triose phosphate is oxidized to phosphoglyceric acid. In intact erythrocytes under the same conditions, only as much glucose is phosphorylated as phosphoglyceric acid is formed, the total amount of phosphorylation being much smaller than in the same quantity of hemolysed blood. All evidence available indicates that the inhibi-

tion of phosphorylation in intact cells is due to the higher concentration of cozymase in them. In fact, the addition of 0.0005 M cozymase to the hemolysate causes a strong diminution of the phosphorylation of glucose; the amount of phosphorylated glucose becomes equal to the amount of phosphoglyceric acid, as is the case in intact cells.

The mechanism of this phenomenon of inhibition is discussed, and the conclusion is reached that the ratio cozymase: dihydrocozymase is the factor controlling the rate of phosphorylation in cells; the oxidized form inhibits, the reduced form accelerates the transphosphorylation with glucose.

Pantothenic acid in the metabolism of Proteus Morganii. ALBERT DORFMAN, SAM BERKMAN and STEWART A. KOSER. *Dept. of Bacteriology and Parasitology, University of Chicago, Ill.* The addition of pantothenic acid to *Proteus Morganii* cells grown on a medium deficient in pantothenic acid markedly stimulates the oxidation of lactic and pyruvic acids. Both substrates are oxidized to acetic acid and carbon dioxide.

To determine more exactly the reaction in which pantothenic acid is involved, a comparison of certain metabolic properties of intact and dried cells was made. Drying was found to result in four important metabolic changes: 1, dried cells in contrast to intact cells are unable to oxidize pyruvic acid; 2, dried cells in contrast to intact cells produce pyruvic acid from lactic acid; 3, dried cells in contrast to intact cells do not produce acetic acid from lactic acid, and 4, the oxidation of lactic acid by dried cells in contrast to intact cells, is stimulated little if at all by pantothenic acid.

This evidence indicates that lactic acid is oxidized in this organism by way of pyruvic acid and that the enzyme(s) responsible for the oxidation of pyruvic acid is destroyed by drying.

It can therefore be concluded that the pantothenic acid stimulation of the oxidation of lactic acid and pyruvic acid is due to the participation of pantothenic acid in the oxidation of pyruvic acid to acetic acid and carbon dioxide.

Combustion of glucose, lactate, pyruvate and non-carbohydrate material by brain tissue. K. A. C. ELLIOTT, D. B. MCNAIR SCOTT and B. LIBET. *Inst. of the Pennsylvania Hospital, Philadelphia.* Measurements of oxygen uptake, CO_2 evolution, and the formation or disappearance of glucose, total carbohydrate, lactate, pyruvate and acetate, have been made on suspensions of rat whole brain in isotonic medium and balance sheets have been drawn up.

In the absence of added substrate, traces of lactate originally present are consumed but about 75 per cent of the respiration occurs at the expense of non-carbohydrate material; the R.Q. is 0.86. In the presence of glucose, the total oxygen uptake over 2 hours is about doubled and all non-carbohydrate oxidation appears to be suppressed since carbohydrate metabolism can account for the entire respiration; the R.Q. is 0.93. Lactate gives about the same respiration rate as glucose but lactate utilization accounts for only about 80 per cent of the respiration; the R.Q. with lactate is 0.88. Pyruvate gives a greater respiration rate than do glucose or lactate and its utilization can account for the entire respiration; the R.Q. with pyruvate is 1.21. Acetate is not utilized but small amounts of a substance estimated as acetic acid are formed when brain tissue respires with or without added glucose or lactate, and added pyruvate gives rise to the formation of an amount of acetate equivalent to about 12 per cent of the pyruvate disappearing. No synthesis of carbohydrate was observed. Low concentrations of pyruvate or lactate are not as effective as low concentrations of glucose in supporting the full rate of respiration.

Some effects of the level of dietary protein on carbohydrate and protein metabolism. GORDON H. ELLIS and L. A. MAYNARD. *Laby. of Animal Nutrition, Cornell Univ., Ithaca, N. Y.* In studies with albino rats a diet containing 10 per cent of protein was compared with a similar one in which the protein level was raised to 41 per cent with a corresponding withdrawal of carbohydrate. A study was made of uric acid, urea and protein of the blood, and of fat and glycogen in the liver. It was found that the level of urea in the blood depends on the level of dietary protein and that different proteins such as casein and liver fed at the same level can result in different levels of blood urea. It was also found that liver glycogen was high on the low protein diet and low on the high protein diet. Glucose tolerance tests indicated that a high level of dietary protein leads to a decreased ability of the animal to handle large doses of glucose.

The observation was made that the shape of a glocose tolerance curve is related to the dose of glucose used in the test. Under favorable conditions our strain of rats (Yale) did not show differences in glucose tolerance that have been reported from other laboratories for this strain.

The effect of sulfapyridine on nicotinic acid metabolism. C. A. ELVEHJEM, L. J. TEPLY and A. E. AXELROD. *Dept. of Biochemistry, Univ. of Wisconsin, Madison.* West (Proc. Soc. Exper. Biol. and Med. **46**: 369, 1941) has shown that sulfapyridine inhibits the curative effect of nicotinic acid in canine black tongue and that raw liver is active under similar conditions. These results have been verified and studies have been made to identify the substance in the fresh liver. The growth response of *L. arabinosis* on the Snell-Wright

medium to nicotinic acid was inhibited by 1γ of sulfapyridine per cubic centimeter of medium. This inhibition was counteracted by para-aminobenzoic acid, by dry liver extract, and by concentrates from liver extract which were low in para-aminobenzoic acid. In spite of the presence of these factors in whole liver, which undoubtedly react with sulfapyridine, we have found certain samples of fresh liver to give a greater growth response in the presence of sulfapyridine than an equivalent amount of dry liver. Low levels of cozymase were inactive but these same levels became active after digestion with Schlenk's enzyme which is known to liberate nicotinamide nucleoside from cozymase. These results indicate that sulfapyridine may act by preventing the formation of the nucleoside from nicotinic acid and that fresh liver functions by supplying the nucleoside.

Assimilation of radioactive carbon from $C^{11}O_2$ by various tissue preparations. E. A. Evans, Jr., Louis Slotin and Birgit Vennesland. *Dept. of Biochemistry, Univ. of Chicago, Ill.* The assimilation of carbon dioxide, as measured by the fixation of C^{11} has been studied in a variety of tissues and tissue preparations, with a view to determining the nature of the reactions involved.

It has been reported previously (E. A. Evans, Jr. and Louis Slotin. J. Biol. Chem. **141:** 439, 1941) that minced pigeon liver in Krebs' bicarbonate saline to which are added pyruvate and malonate, will assimilate about 30 per cent of the added carbon dioxide in 40 minutes. Under the same conditions a similar suspension of rat liver gives identical results as does also a suspension of fresh beef liver. With the latter, under anaerobic conditions (95 per cent N_2, 5 per cent CO_2), the amount of carbon dioxide assimilated is approximately halved.

The presence of intact cells is not necessary for the reaction. A liver extract prepared by grinding liver briefly with phosphate saline in a Waring mixer and centrifuging off the heavy particles shows active assimilation.

A phosphate extract of acetone-dried beef liver likewise assimilates carbon dioxide, but the amount of C^{11} fixed is very small. Control experiments with boiled tissue preparations give uniformly negative results.

The excretion of iodine by the salivary and gastric glands. James Flexner, Maurice Bruger and Samuel Member. *Medical Research Laby., New York Post-Graduate Medical School and Hospital, Columbia Univ., New York City.* The excretion of iodine by the salivary and gastric glands following the intravenous injection of sodium iodide was studied in 5 subjects as follows: the individual was placed on his right side with the head at a lower level than the stomach. A metal gag was placed between the teeth in order to prevent the swallowing of saliva. After control specimens of saliva (by drooling) and gastric juice (by Levine tube) were obtained, 0.5 mgm. of histamine and 12.5 mgm. of acetyl-B-methylcholine chloride were injected subcutaneously in order to stimulate gastric and salivary secretions, respectively. One cubic centimeter of an aqueous solution of sodium iodide containing 93 mgm. of sodium iodide (78.8 mgm. of iodine) was then injected intravenously. Specimens of saliva and gastric juice were then collected at frequent intervals for one hour. The results obtained were as follows, expressed in milligram per cent (average for the 5 experiments): Salivary Iodine: Control, 0.025; 20 min., 4.830; 30 min., 5.274; 35 min., 3.530; 40 min., 4.464; 60 min., 9.621. Gastric Iodine: Control, 0.039; 15 min., 1.040; 20 min., 3.429; 30 min., 6.440; 40 min., 4.279; 60 min., 2.436.

Conclusion: Inorganic iodine is selectively concentrated and secreted by the salivary and gastric glands.

Phosphate compounds labeled by radio-phosphorus in working and resting muscles of rats. Eunice V. Flock and Jesse L. Bollman. *Inst. of Experimental Medicine, Rochester, Minn.* Radioactive phosphorus as sodium phosphate was injected intravenously into rats. At various intervals later one leg was stimulated three times each second. Analyses of phosphorus content and radioactivity were made for inorganic phosphate, phosphocreatine, adenosine triphosphate, and hexose monophosphate in the stimulated leg and in the unstimulated leg taken at the same time. Most of the work periods were taken while the specific radioactivity was several times greater in the inorganic phosphate than in the other compounds. The greatest change of specific activity occurred in the dilution of radioactivity of the inorganic phosphate due to the hydrolysis of phosphocreatine and adenosine triphosphate of lower specific activity. During continued work the uptake of radio-phosphorus by the phosphocreatine, adenosine triphosphate, and hexose monophosphate was similar to that which was occurring in the resting leg.

Periods of work and rest were alternated, one minute of work (180 contractions) followed by four minutes rest followed by five or more similar periods of one minute work and four minutes rest. In this way hydrolysis and resynthesis of large amounts of phosphocreatine, and adenosine triphosphate were accomplished. The specific activities of these compounds were not greatly altered from those of the resting leg. These observations indicate that the phosphoric acid hydrolyzed during muscular activity diffuses but slowly through the muscle and does not mix readily with the inorganic phosphate previously there.

Resynthesis of phosphocreatine and adenosine triphosphate during rest recombines almost entirely the original phosphate of those compounds.

Brain cephalin, a mixture of phosphatides. JORDI FOLCH. *Hospital of The Rockefeller Inst. for Medical Research, New York City.* Brain cephalin (J. L. W. Thudichum, Physiological chemistry of the brain, London, 1884) is not a single substance but a mixture of amino phosphatides (phosphatides having all of their N as NH_2). It can be separated into three fractions of different chemical composition by the following method:

A solution of 8 per cent by weight of ox brain cephalin in $CHCl_3$ is prepared. To it is added 1.1 as much absolute alcohol as $CHCl_3$ by volume. A precipitate forms (fraction I) and is collected. To the supernatant is added from 4 to 5 times as much alcohol as in the previous operation, giving another precipitate (fraction II). The filtrate is concentrated to a small volume, and poured into acetone, giving another precipitate (fraction III).

Fraction I has inositol as a constituent (J. Folch and D. W. Woolley. Unpublished results). Inositol is absent from the other fractions. Fraction II is for the most part phosphatidyl-serine (J. Folch and H. Schneider. J. Biol. Chem. **137**: 51, 1941; J. Folch, J. Biol. Chem. **139**: 973, 1941); it has a low iodine number and its P can be isolated as glycerophosphoric acid. Fraction III represents a more unsaturated compound (I.N.= 80) and contains no amino acid. Most likely it has ethanolamine as its NH_2-bearing group.

Studies on continued alkali ingestion in man. ALFRED H. FREE, DEAN F. DAVIES, GORDON E. GUSTAFSON and VICTOR C. MYERS. *Dept. of Biochemistry, School of Medicine, Western Reserve Univ., Cleveland, O.* Sodium citrate in the amount of 0.18 gram per kilogram body weight per day, or an equivalent amount of sodium bicarbonate, was ingested by a series of normal individuals. Each subject received each compound in two series of experiments, each period ranging from six to eight days. One half of the alkali was ingested in the morning and four hours thereafter a blood sample was obtained. The remaining alkali was taken four hours later. Serum total base, serum CO_2 content, serum pH and serum chloride determinations were made. Acid-base balance studies on the urine were also carried out. No marked changes in the acid-base balance of the blood were observed but a small, consistent increase in serum CO_2 content and a reciprocal decrease in serum chloride were noted. The effect of the citrate was similar to that of the bicarbonate.

Two urea clearance studies were carried out on separate days at the beginning and end of each experimental period. No significant change was observed as a result of the alkali ingestion.

Studies on the absorption and metabolism of galactose and glycine in dogs with vitamin B deficiency. ALFRED H. FREE, JACK R. LEONARDS and VICTOR C. MYERS. *Dept. of Biochemistry, School of Medicine, Western Reserve Univ., Cleveland, O.* Intestinal absorption was measured under physiological conditions by following the blood levels and excretion of galactose or glycine administered by stomach tube. In the case of galactose the rate of metabolism was measured by following the decrease of blood galactose after intravenous injection of comparable amounts of the sugar. The metabolism of glycine was measured by the increase in blood urea and the excretion of urea in the urine.

The intestinal absorption of adult dogs on a purified diet deficient in vitamin B complex supplemented with yeast was measured. The animals were then rendered deficient by removing the yeast component of the diet. After four to six weeks, typical symptoms of vitamin B_1 deficiency developed and the aimals which were not given vitamin B_1 supplements died. Absorption measurements were carried out during the depletion period and at the time acute symptoms were observed. Small but consistent decreases in both rate of absorption and metabolism were noted during the depletion period. These changes became greater when acute symptoms appeared. Gastric emptying time was also greatly decreased in the acute stage of the deficiency. [*Aided by a grant from the Josiah Macy, Jr. Foundation.*]

The determination of thiamin in blood. THEODORE E. FRIEDEMANN and THADDEUS C. KMIECIAK. *Dept. of Physiology and Pharmacology, Northwestern Univ. Medical School, Chicago, Ill.* Procedure: Lake 5 cc. of blood in a tube calibrated at 50 cc. Add 0.5 cc. of *N* HCl, and approximately 0.2 g. of Clarase. Incubate for 1 hour at 37 to 45°. Add 2 cc. more of *N* HCl. Heat 10 minutes, stirring frequently, in a boiling water bath. Cool, add 10 cc. of fresh 10 per cent HPO_3, adjust to mark, and centrifuge. Emulsify the precipitate with 5 cc. of HPO_3 and again adjust to mark. Pass the warmed combined clear extracts through a Hennessy column containing Decalso. Wash twice with 5 cc. of hot water. Elute with 25 per cent NaCl (containing 2 cc. of concentrated HCl per 1) until 25 cc. are collected. Prepare a reagent blank.

To 10 cc. aliquots of extract contained in Hennessy reaction vessel, add rapidly and with mixing 6 cc. of oxidation mixture (100 cc. of 40 per cent NaOH and 10 cc. of 1 per cent $K_3Fe(CN)_6$) and 13 cc. of iso or normal butyl alcohol. Shake 90 seconds, centrifuge, and dehydrate the supernatant solution with 3 small scoops full of anhydrous Na_2SO_4. Determine the fluorescence.

Duplicate analyses agree within ±0.5 micrograms per 100 cc. *Samples must be kept cold and analyzed within 48 hours of collection.* Results of analysis of 48 samples from 26 normal subjects, expressed in micrograms per 100 cc., were: extreme range, 4.0 to 10.3; arithmetic mean, 5.96; standard deviation, 1.54. [*Aided by the Clara A. Abbott Fund of Northwestern University.*]

The excretion of thiamin. THEODORE E. FRIEDEMANN and THADDEUS C. KMIECIAK. *Dept. of Physiology and Pharmacology, Northwestern Univ. Medical School, Chicago, Ill.* Contrary to accepted belief, from 10 to 20 per cent of the thiamin in freshly voided urine is present as cocarboxylase (increase of isobutyl alcohol-soluble thiochrome after treatment with phosphatase).

Determinations of the concentration of thiamin in the blood and the rate of its excretion in the urine have given calculated clearance values as high as those obtained with diodrast. Whole blood clearance values varying from 850 to 1180 cc. per minute have thus been obtained from human subjects receiving from 40 to 50 mgm. of thiamin intravenously. The increase of thiamin in the blood varied from 5 to 60 micrograms per 100 cc., and the flow of urine varied from 6.3 to 13.4 cc. per minute.

A comparison of the data in the literature indicates similar high clearances for riboflavin and pyridoxine. As far as we are aware, this is the first recorded instance of maximum clearances obtained after the injection of a normal and necessary constituent of the animal body. [*Aided by the Clara A. Abbott Fund of Northwestern University.*]

The metabolism of pyruvic acid. THEODORE E. FRIEDEMANN, S. C. WERCH and GLADYS E. HAUGEN. *Dept. of Physiology and Pharmacology, Northwestern Univ. Medical School, Chicago, Ill.* Solutions of sodium pyruvate and sodium d-lactate (0.56 M), containing 0.6 per cent NaCl, were injected intravenously into anesthetized (Nembutal) dogs and unanesthetized rabbits kept under approximately basal conditions (S. C. Werch. J. Lab. Clin. Med. **26**: 725, 1941). The animals received 5.6 mM per kilo of body weight. Sodium acetate (0.56 M) and sodium chloride (0.9 per cent) were used in control experiments. Lactic and pyruvic acids were determined in blood filtrates. The filtrates were allowed to react from 5 to 60 minutes with 2,4-dinitrophenylhydrazine, and the resulting hydrazones, after extraction and alkalinization, were examined with the Evelyn colorimeter, using the 420 and 520 filters.

In the control experiments, no significant rise either of lactic or pyruvic acid was observed. In some animals, large quantities of lactic acid were produced during and immediately after the injection of pyruvate, and the normal lactic-pyruvic ratio was attained in about the same time as after severe exercise. In most of the animals, besides lactic acid, the blood contained large quantities of α-ketoglutaric acid and other material which reacted more slowly than α-ketoglutaric acid. Injected lactate resulted in the production of large quantities of pyruvic acid. The slow-reacting material and α-ketoglutaric acid were not apparently produced, nor were they present in the blood in demonstrable quantities after exercise.

It would appear that α-ketoglutaric acid is not formed in significant quantities during the normal metabolism of the intact tissues. [*Aided by the Clara A. Abbott Fund of Northwestern University.*]

The reaction of formaldehyde with amino acids. E. H. FRIEDEN, M. S. DUNN and C. D. CORYELL. *Dept. of Chemistry, Univ. of California, Los Angeles.* The polarimetric method of investigation of formaldehyde-amino acid complex formation, previously reported for proline (E. H. Frieden, M. S. Dunn and C. D. Coryell. J. Physiol. Chem. (in press)), has been extended to the amino acids leucine and histidine and the imino acid methyl leucine. The high concentrations of formaldehyde employed in some cases required an estimation of solvent effects. In the case of leucine, the corrections were determined by comparative studies of the effect of formaldehyde on the rotations of dimethyl leucine in alkaline solution and of leucine, monomethyl leucine, and dimethyl leucine in acid solution.

The physical mechanisms postulated by Levy (M. Levy, J. Biol. Chem. **99**: 767, 1932–33) for the leucine-formaldehyde reactions have been completely confirmed; however, those proposed for histidine have been shown to be incomplete. The polarimetric method of determining equilibrium constants for the reactions of basic amino acids with formaldehyde appears to be superior to the electrometric procedure.

Simplified bromide determination for extracellular fluid measurement. MAX M. FRIEDMAN. *Dept. of Pathology, Fordham Hospital, New York City.* The estimation of bromide has become of interest because of its use in the measurement of extracellular fluid volume. The fusion method of Brodie and Friedman (J. Biol. Chem. **124**: 511, 1938), while accurate, is not simple enough for routine use. By substituting a trichloracetic acid filtrate of serum for the fusion, the method is simplified without loss of accuracy.

Treat 1 part of serum with 5 parts of 10 per cent trichloracetic acid and buffer 10 ml. of filtrate with 5 ml. of 40 per cent $NaH_2PO_4 \cdot H_2O$. Oxidize the bromide to bromate by adding 8 ml. of N NaOCl and heating on the water bath for 10 minutes. Destroy the excess hypochlorit by heating with 5 ml. of 50 per cent sodium formate for 5 minutes more, cool, and acidify with 10 ml. of 6 N H_2SO_4.

Add 1 ml. of 20 per cent KI and 3 drops of 10 per cent ammonium molybdate, and titrate with 0.005 N thiosulfate. A reagent blank is subtracted from each determination. Bromide added to serum is quantitatively recovered and analyses of unknown sera check with the fusion method.

At the plasma concentrations encountered the bromide excretion is usually small and may be neglected. When it must be known the halides are first separated as the silver salts from interfering substances in the urine. The silver salts are suspended in phosphate buffer and oxidized with NaOCl, converting AgBr to the soluble bromate. After filtering off the AgCl the bromate is determined as above.

The specificity of chymotrypsin. JOSEPH S. FRUTON and MAX BERGMANN. *Labs. of The Rockefeller Inst. for Medical Research, New York City.* The specificity of a proteolytic enzyme may be characterized through a determination of the kinetics of the hydrolysis of peptide linkages in a number of substrates. The ratio of the first-order velocity constants for two substrates of closely related structure gives quantitative expression to the influence of their structural differences on the rate of hydrolysis. It has been found that several different proteolytic enzymes give similar ratios for the hydrolysis of two given substrates. This and other results have provided the basis for a revised classification of proteolytic enzymes (J. S. Fruton, G. W. Irving and M. Bergmann. J. Biol. Chem. **141**: 763, 1941). The position of chymotrypsin in this classification has now been investigated. This enzyme hydrolyzes an extensive series of synthetic substrates of low molecular weight. Among these are glycyl-*l*-tyrosine amide, glycyl-*l*-phenylalanine amide, *l*-tyrosine amide, *l*-phenylalanine amide, *l*-tyrosylglycine amide and *l*-phenylalanyglycine amide. The kinetics of hydrolysis of the above substrates have been determined for twice and four times crystallized chymotrypsin and for crystalline γ-chymotrypsin. Although the presence of the aromatic side chain of tyrosine or phenylalanine appears to be essential for chymotrypsin action, this enzyme does not exhibit the same specificity as pepsin or cathepsin I which require the same side chains in their substrates.

Photochemical production of hydrogen in green algae. HANS GAFFRON and JACK RUBIN. *Dept. of Chemistry, Univ. of Chicago, Ill.* Progress in the field of photosynthesis has been slow as compared with advances in the fields of respiration and fermentation because photosynthesis appeared to be a chemically rigid mechanism unlikely to be separated into its partial reactions. Two years ago it was found that in some green algae the evolution of oxygen in photosynthesis could be replaced by an equivalent absorption of molecular hydrogen. This showed definitely that the evolution of oxygen was a partial reaction not necessarily connected with the photochemical reduction of carbon dioxide. Further investigations as to the connections between the system transferring molecular hydrogen (hydrogenase) and the photochemical process proved that the former can react independently; it liberates as well as absorbs hydrogen in the dark. These algae produce hydrogen by fermentation like many non-photosynthetic bacteria. Yet the connection between the hydrogenase and the chlorophyllous system is such that upon illumination under nitrogen and in absence of the normal substrate, carbon dioxide, the algae liberate molecular hydrogen at a rate about ten times that of the dark fermentation. This new photochemical reaction depends on the presence of intracellular or added hydrogen donors (e.g. glucose), but it is quite different from the dark fermentation. The fermentation can be inhibited by specific poisons while the photochemical reaction continues. The importance of these results arises from the fact that they seem to indicate that the photochemical hydrogen transport during photosynthesis and the reduction of carbon dioxide are separable partial reactions.

Oxalic acid hydrolysate of casein for bacteriological purposes. L. E. GILSON, A. A. TYTELL, M. A. LOGAN and PATRICIA PERKINS. *Dept. of Biological Chemistry, Univ. of Cincinnati Medical College, Cincinnati, O.* Casein is hydrolysed by autoclaving with a solution of oxalic acid. The resulting amino acid mixture is superior to hydrolysates at present available for growth, toxin production, and vitamin assays using several bacterial species. Preparation involves but few manipulations and reagents.

Method: 100 grams of casein (technical or vitamin-free) are placed in a flask with 150 grams of oxalic acid and 300 cc. of distilled water. The mixture is warmed and shaken until all lumps are broken up and most of the oxalic acid is dissolved, then autoclaved 40 hours at 15 pounds. The biuret test is now usually negative. After cooling to 4°C., oxalic acid which crystallizes out is filtered off, the filtrate is made slightly alkaline with magnesia-free calcium hydroxide, calcium oxalate is filtered off, excess calcium is precipitated with ammonium carbonate and filtered off, and the filtrate is freed of ammonia by boiling or evaporation. Iron may be removed if desired by the method of Mueller or by precipitating the excess calcium with phosphate instead of carbonate.

This hydrolysate was found highly satisfactory for growth and toxin production with Cl. welchii, growth of *Lactobacillus casei* ϵ (for pantothenic acid assay), and for several species of *Proteus*.

The heterophile antigen of pneumococcus. WALTHER F. GOEBEL, MARK H. ADAMS and THEODORE SHEDLOVSKY. *Hospital of The Rockefeller Inst. for Medical Research, New York.*

The isolation of substances with heterophile properties from mammalian tissue and from Gram negative microorganisms has been described by numerous investigators. The characterization and isolation of similar substances from the Gram positive group of microorganisms has not as yet been achieved. A purified antigenic substance endowed with heterophile properties has been recovered from autolysates of the R variant of type I Pneumococcus. The substance occurs in very small quantities in the bacterial cells. Some 700 mgm. can be isolated from the bacteria obtained from five hundred liters of culture. The antigen is a non-dialyzable water soluble lipo-carbohydrate complex constituted from molecules of an acetylated amino sugar, an unidentified hexose, phosphoric acid, and a lipid. The polysaccharide-lipid complex is fully antigenic and stimulates the production of precipitating and hemolytic antibodies when injected into rabbits. The heterophile antigen is intimately related to the "C" or species specific carbohydrate of pneumococcus. It differs from the latter, however, in certain of its physical, chemical and immunological properties. The antigen appears to be a distinct chemical entity, for numerous preparations show constant analytical properties and the derivative migrates as a homogeneous component when examined by electrophoresis. In quantities as small as two micrograms the antigen inhibits the lysis of sheep cells by homologous antiserum in the presence of complement. Unlike analogous substances derived from Gram negative microorganisms, the pneumococcus lipo-carbohydrate shows no toxic effect when injected either into rabbits or mice.

Ribonuclease and thymonucleodepolymerase. JESSE P. GREENSTEIN. *National Cancer Inst., National Inst. of Health, U. S. Public Health Service, Bethesda, Md.* The enzyme which depolymerizes native thymonucleic acid, thymonucleodepolymerase, is found in high concentration in the pancreas. The enzyme which depolymerizes the yeast type of nucleic acid, ribonuclease, has been isolated from the pancreas by Kunitz.

Native sodium thymonucleate (Hammarsten) which in 0.5 percent aqueous solution possesses a relative viscosity of 51.1 at 30° and 16.0 cm. H_2O pressure is reduced under the same conditions to a relative viscosity of 1.2 in fifteen minutes on treatment with dilute pancreas extract. Addition of crystalline ribonuclease, up to 8 mgm. of protein per cubic centimeter, to such solutions of native thymonucleate results in no more depolymerization of the nucleate than is accomplished by the addition of an equivalent amount of some inert protein such as horse serum albumin. It seems probable that each type of nucleic acid requires a different enzyme for the preliminary degradation of the molecule.

Thymonucleodepolymerase is found not only in animal but in plant tissues as well. Extracts of the embryos of the following plant seeds exert a strong depolymerizing effect on native sodium thymonucleate: corn, wheat, pumpkin, sunflower, and lima bean. It would appear that both thymonucleic and ribosenucleic acids, as well as their specific depolymerases, are distributed in animal and in plant tissues, and that the former distinction between "plant" and "animal" nucleic acids is no longer tenable.

The effect of pH and of the amount of solid phase upon the solubility of calcium phosphate. ISIDOR GREENWALD. *Dept. of Chemistry, New York Univ. College of Medicine, New York City.* The solubility of calcium phosphate, expressed as $[Ca^{++}]^3\ [PO_4^{\equiv}]^2$, increases with the amount of solid phase and with the pH of the solution. This is in accord with the view that "between dicalcium phosphate and lime, there exists, in the ternary system, a continuous series of solid solutions having an apatite lattice" (S. Eisenberger, A. Lehrman, and W. D. Turner. Chemical Reviews **26**: 257, 1940). The contrary results of Logan and Taylor (M. A. Logan and H. L. Taylor. J. Biol. Chem. **119**: 293, 1937) are believed to be due to inadequate equilibration.

Some new compounds of zinc and glycine. ISIDOR GREENWALD. *Dept. of Chemistry, New York Univ. College of Medicine, New York City.* Mixtures of glycine and zinc chloride have a greater buffer capacity between pH 2 and 11 than the sum of those of $Zn\ Cl_2$ and glycine. The results indicate the existence of a series of complexes which have been formulated as:

$$[Zn(^{+}H_3NCH_2COOH)]^{+++},$$

$$[Zn(^{+}H_3NCH_2COO^{-})]^{++},$$

$$\left[Zn(^{+}H_3NCH_2COO^{-})\begin{matrix}OH\\ \end{matrix}\right]^{+},$$

$$\left[Zn(^{+}H_3NCH_2COO^{-})_2\begin{matrix}OH\\OH\end{matrix}\right],$$

$$\left[Zn(^{+}H_3NCH_2COO^{-})_3\begin{matrix}O\\OH\end{matrix}\right]^{-} \text{ and}$$

$$\left[Zn(^{+}H_3NCH_2COO^{-})_4\begin{matrix}O\\O\end{matrix}\right]^{-},$$

The following new compounds have been prepared:

$$[Zn(^{+}H_3NCH_2COOH)_2]Cl_4\cdot H_2O,$$

$$[Zn(^{+}H_3NCH_2COO^{-})_3]Cl_2,$$

$$\left[Zn(^{+}H_3NCH_2COO^{-})_3\begin{matrix}O\\OH\end{matrix}\right] Na, \text{ and}$$

$$\left[Zn(^{+}H_3NCH_2COO^{-})\begin{matrix}O\\O\end{matrix}\right] Na_2\cdot H_2O$$

See p. 201 for correction

The intermediary metabolism of alanine containing C^{13}. S. GURIN and D. WRIGHT WILSON. *Dept. of Physiological Chemistry, Univ. of Pennsylvania Medical School, Philadelphia.* Alanine containing excess C^{13} in the carboxyl group was prepared from sodium cyanide containing an excess of C^{13}. The isotopic alanine was injected into phlorhizinized rats and the excreted glucose isolated. After 24 hours, the animals were killed and various body constituents were isolated. A small excess of C^{13} was found in the urinary glucose. The direct quantitative conversion of alanine to glucose in the phlorhizinized rat does not occur.

Oxidation-reduction potential requirements and O_2 consumption of obligate anaerobes. MARTIN E. HANKE, JOHN H. BAILEY and YALE J. KATZ. *Dept. of Biochemistry, Univ. of Chicago, Ill.* An electrolytic method for controlling the oxidation-reduction potential of aqueous solutions has been devised. For four obligate anaerobes, on a glucose nutrient broth medium at pH 6.6, the following limiting E_h values have been found to distinguish growth from no growth: *Clostridium histolyticum*, 0.090 volt; *Clostridium sporogenes* and *Bacteroides vulgatus*, 0.150 volt; *Clostridium perfringens* (*welchii*), 0.165 volt. At higher pH values, 7.0 to 7.4, the limiting potential for all four organisms is more negative by 20 to 50 millivolts, and for *Cl. sporogenes* at pH 6.2 the limiting potential is also more negative than at pH 6.6, indicating a maximum limiting potential at 6.6. These limiting potentials are independent of variations in O_2 tension. With adequate negative electrolysis so the potential is kept below the limiting value, these organisms will grow in a continuous current of air. Measurement of O_2 consumption during growth at controlled E_h has shown that when *Bacteroides vulgatus* grows in the presence of O_2, O_2 is consumed with a minimal Q_{O_2} of 11.

Electrolyte and water exchange between skeletal muscle, "available (thiocyanate) fluid," and plasma in the dog following the administration of desoxycorticosterone acetate. DAVID M. HARKNESS, EDWARD MUNTWYLER, ROBERT C. MELLORS and FREDERICK R. MAUTZ. *Depts. of Biochemistry and Surgery, School of Medicine, Western Reserve Univ., Cleveland, O.* Electrolyte and water content of skeletal muscle and plasma, plasma volume (use of dye T-1824), and "available (thiocyanate) fluid" were studied in dogs, maintained on a high sodium-low potassium diet, before and following the subcutaneous injection of desoxycorticosterone acetate.

The majority of animals receiving desoxycorticosterone acetate (1 mgm. per kgm. daily) for periods of two weeks exhibited significant increases of plasma volume and "available fluid", averaging 14 and 16 per cent, respectively. The composition of the plasma showed little change, aside from a fall of potassium to as low as 50 per cent of the control level.

Skeletal muscle analyses revealed an appreciable gain of sodium and fall of potassium, per Kg. of fat-free muscle. The chloride remained unchanged, while the water content was reduced slightly. Calculations made in the customary manner revealed the following: *a.* There was a fall of intracellular potassium, per Kg. of intracellular water, which was in large part compensated by a gain of intracellular sodium. *b.* Usually there was an actual loss of total intracellular sodium plus potassium. *c.* The majority of the experiments showed a slight decrease in the water content per kilogram of muscle cells. Calculations of the changes of the muscle phases relative to the control revealed that on the average there was an absolute decrease of the muscle per Kg. of control muscle, which consisted of a decrease in the intracellular phase and a slight increase in the extracellular phase. [*Aided by a grant from the John and Mary R. Markle Foundation.*]

Destruction of angiotonin by extracts of various tissues. O. M. HELMER, K. G. KOHLSTAEDT and IRVINE H. PAGE. *Lilly Laby. for Clinical Research, Indianapolis City Hospital, Indianapolis.* Extracts of kidney, muscle, liver and intestinal mucosa were tested for their activity in destroying or neutralizing angiotonin *in vitro*. The extracts were incubated with angiotonin at 37°C. for thirty minutes, boiled, and the residual pressor activity of the filtrates determined in pithed anesthetized cats.

With regard to the effect of pH, muscle, liver and extracts of intestinal mucosa showed greatest activity between pH 7 and pH 8. This difference was especially marked with muscle extracts. These yielded at pH 7.0 only 5 per cent recovery of the pressor activity of a standardized solution of angiotonin, whereas 74 per cent remained in the filtrates after incubation at pH 4.0. In contrast with these results, extracts of kidney showed maximum activity at pH 4.0.

The differences in pH maxima of tissue extract activity suggest that the anti-angiotonin activity of kidney depends upon a different system from that of muscle, liver and intestinal mucosa.

The assay of anti-pressor extracts of kidney by in vitro destruction of angiotonin. O. M. HELMER, K. G. KOHLSTAEDT, G. F. KEMPF and IRVINE H. PAGE. *Lilly Laby. for Clinical Research, Indianapolis City Hospital, Indianapolis.* The use of hypertensive dogs and rats for assay of anti-pressor extracts of kidney is time consuming, and large amounts of extract are needed. Furthermore, differences in the response of individual animals makes the interpretation of the results difficult.

In the proposed method 1 cc. of extract equivalent to 12.5 to 100 mgm. of original kidney is

mixed with 1 cc. of fiftieth molar acetate buffer at pH. 4.0, and incubated at 37° for thirty minutes with 0.5 cc. of standardized angiotonin. The reaction is stopped by immersion in boiling water for ten minutes. Boiling also destroys any renin that is present in kidney extracts. The pressor response of an aliquot of the test solution is compared to that of untreated angiotonin by intravenous injection into a pithed cat.

By means of this assay it has been found that most of the material in anti-pressor kidney extracts which destroys angiotonin is precipitated between 0.3 and 0.6 saturation with ammonium sulfate at pH 4.0. This is the same range of ammonium sulfate precipitability that was found for the anti-pressor activity of kidney extracts assayed on hypertensive dogs and rats.

The results of the two methods of assay will be presented.

The use of heavy carbon as a tracer element. ALLAN HEMINGWAY, MARIAN SWENDSEID and A. O. NIER. *Dept. of Physiological Chemistry, The Medical School, Univ. of Minnesota, Minneapolis.* Heavy methane (excess C^{13}) has been concentrated in a thermal diffusion column with an increase in the concentration of C^{13} of 10 to 19 fold. Heavy carbon dioxide formed by methane combustion has been used for metabolism studies. Analyses for C^{13} abundance have been made with a mass spectrometer.

The rate of elimination of heavy carbon dioxide from rats and mice after injections of $NaHCO_3$ (excess C^{13}) have been measured with single and repeated injections of $NaHCO_3$. In other experiments the animals have been placed in an atmosphere of heavy carbon dioxide (5 per cent) for 24 hours and the incorporation of environmental CO_2 into organic compounds measured. Only a slight uptake of CO_2 has been found. In addition the natural abundance of C^{13} in animal tissues has been measured. These experiments have been designed as control experiments to determine the usefulness of heavy carbon as a tracer element in metabolism.

Canine cystinuria. The cystine output on an arachin diet. W. C. HESS and M. X. SULLIVAN. *Chemo-Medical Research Inst., Georgetown Univ., Washington, D. C.* We have previously reported the effect of feeding a diet containing various amounts of casein and the addition thereto of cysteine and methionine on the excretion of cystine by two cystinuric dogs (W. C. Hess and M. X. Sullivan, Abstracts American Chemical Society, Sept. 8, 1941). Casein is a protein relatively rich in methionine and low in cystine. We have now substituted a protein, arachin, low in methionine and rich in cystine for the casein in the diet. At two protein levels the arachin diet caused the excretion of less cystine than did the corresponding levels of casein. The addition of both methionine and cysteine resulted in the excretion of extra urinary cystine. However the feeding of cysteine was more effective in stimulating cystine output than was the feeding of methionine. Data will be presented showing the relation of cystine excretion to the cystine and methionine content of the arachin fed.

Isolation of a new triol from the urine of a patient with an adrenocortical carcinoma. H. HIRSCHMANN. *Dept. of Obstetrics and Gynecology and the Gynecean Hospital Inst. of Gynecologic Research, Univ. of Pennsylvania, Philadelphia.* The urine of a boy with a carcinoma of the adrenal cortex and metastases in liver and lungs was promptly hydrolyzed and extracted with ether. The neutral fraction of this extract was unusually large (about 3.2 gms. per liter of urine). From this material a benzene-insoluble residue was obtained which upon repeated recrystallization yielded a substance melting at 267–270° (corrected). Analytical data on this compound agreed best with the formula $C_{19}H_{30}O_3$. Upon acetylation at room temperature a triacetate ($C_{25}H_{36}O_6$) melting at 187–188.5° was formed. Catalytic hydrogenation (palladium) of the unesterified compound yielded a substance $C_{19}H_{32}O_3$ melting at 256–260°. The original compound was not precipitated with digitonin in 80 per cent ethanol and was unstable towards periodic acid. The properties of the isolated substance demonstrate it to be an unsaturated triol differing from any steroid hitherto isolated from urine or adrenal extracts.

The measurement and calculation of hydrogen ion concentrations in acetate and phosphate buffers. DAVID I. HITCHCOCK and ROZANNE PETERS. *Laby. of Physiology, Yale Univ. School of Medicine, New Haven, Conn.* The accepted pH values for acetate and phosphate buffers require a variation in pK′ of 0.04 to 0.08. Such troublesome variations were avoided by the following procedure. Buffer solutions were prepared containing 0.025 M sodium acetate or disodium hydrogen phosphate, the acidity being varied by hydrochloric acid and the ionic strength being kept constant at 0.16 by the presence of sodium chloride. These solutions were studied by means of glass electrodes, in cells with and without liquid junctions, at 25° and 38°C. By assuming complete ionization in a standard solution of 0.01 M hydrochloric acid in 0.15 M sodium chloride, and constancy of activity coefficients in solutions of constant ionic strength, a value of C_H (not pH) was calculated from each electromotive force measurement. These C_H values were used in the simple mass law equation

$$K_c = C_H C_B / C_A$$

to calculate classical dissociation constants. The resulting K_c values for each buffer acid were

constant within 0.01 in the logarithm, even though the buffer ratio, C_B/C_A, was varied from 1:9 to 9:1. A knowledge of these constants makes possible the preparation of solutions of any desired value of $-\log C_H$, between about 3.5 and 7.6, and of physiological ionic strength. [*This work was aided by a grant from the Fluid Research Funds of the Yale University School of Medicine.*]

Chemical changes in the rabbit heart during hypertrophy. GEORGE H. HITCHINGS and JOSEPH T. WEARN. *Dept. of Medicine, School of Medicine, Western Reserve Univ., Cleveland, O.* Aortic insufficiency, experimentally produced, is followed by a rapid hypertrophy of the myocardium. The chemical changes which occur during the early period of rapid growth are similar to those which take place in tissues stimulated to rapid growth by means of hormones. During the first three days the extracellular phase increases rapidly, both relatively and absolutely. Potassium concentration, per unit of cell solids, is maintained at normal levels but the synthesis of phosphorus compounds is less rapid. Within a week a normal relationship between the phases is reestablished.

The myocardial cells appear to hypertrophy at a more or less constant rate for several weeks, after which further increases in the heart size cannot be distinguished clearly. The hypertrophied hearts are characterized by a high intracellular water content, but several months after operation the concentrations of intracellular electrolytes are essentially normal, when calculated on the basis of a normal cell solid content. At later periods there is a tendency for the potassium concentration of the intracellular phase to fall below normal. [*Aided by a grant from the Commonwealth Fund.*]

A dilution technique for the photoelectric analysis of a mixture of two colors. WILLIAM S. HOFFMAN. *Dept. of Physiological Chemistry, Chicago Medical School, Chicago, Ill.* A new, simple dilution procedure has been developed which allows the analysis, with a photoelectric colorimeter, of the relative proportions of two colored substances in the same solution, without requiring the determination of the absolute concentrations of the two substances. Two light filters are used which transmit light in the region of the greatest absorption by each colored solute. The solution is diluted with the solvent until it reads an arbitrarily fixed value with one filter, and it is then read with the other filter. A smooth curve of values for the various proportions is obtained on semi-logarithmic paper, sensitive in the low percentile range. If a second curve is made with the filters reversed, a complementary curve is obtained, sensitive in the high percentile range. The procedure has been applied successfully to the determination of pH with a two-colored indicator such as methyl red or phenol red. In this determination a drop or two of the indicator solution is diluted with the solution to be tested until it reads the arbitrarily fixed value with one filter and then the solution is read with the other filter. The pH of the urine can be estimated in spite of its intrinsic color, by using the sample of urine instead of water for the 100 setting. The method is also applicable to the determination of relative amounts of methemoglobin or carbon monoxide hemoglobin in a blood sample, provided appropriately narrow light filters are utilized.

An insulin test for differentiating vagal from non-vagal stomach pouches. FRANKLIN HOLLANDER, EDWARD E. JEMERIN and VERNON A. WEINSTEIN. *Labs. of The Mount Sinai Hospital, New York City.* The unreliability of criteria in general use for distinguishing vagal from non-vagal stomach pouches in non-esophagotomized dogs (i.e., various secretory responses to sight, smell, taste, and ingestion of food; see previous abstract) led us to seek a chemical stimulus which would act centrally upon the vagus nerve. Insulin, or rather the hypoglycemia produced by insulin, seemed promising for this purpose, since its effect is abolished by atropinization and by vagus section. Accordingly, we studied the response to insulin hypoglycemia of 8 of the dogs (provided with Pavlov, Heidenhain, or our own vagal pouch) which had been used in the previous study. Insulin sufficient to induce a marked hypoglycemia (55 mgm. per cent or lower) was injected intravenously and the blood sugar level was carefully followed at 30 minute intervals, while samples of gastric secretion were collected at 15 minute intervals. Pouches with different degrees of innervation invariably responded with the secretion of free acid; non-vagal pouches on the contrary secreted no free acid whatsoever, regardless of how low a blood sugar level was obtained. The complete consistency and predictability of response established the validity of insulin hypoglycemia as a criterion for the qualitative determination of pouch vagality. It was impossible, however, to establish any quantitative relation between the magnitude of the insulin dosage or of the hypoglycemia on the one hand, and the volume-rate of secretion or the acidity on the other. [*This investigation was supported in part by a grant from the Friedsam Foundation.*]

Studies of experimental amino acid deficiency in man. I. Nitrogen balance. L. EMMETT HOLT, JR., ANTHONY A. ALBANESE, LANDRUM B. SHETTLES, CHARLOTTE KAJDI and DOROTHY M. WANGERIN. *Dept. of Pediatrics, Johns Hopkins Univ., Baltimore, Md.* Four male human volunteers were given a diet deficient in tryptophane for two to five weeks. Each subject developed a negative nitrogen balance within a few days which persisted

as long as the experimental diet was continued. The addition of tryptophane restored N equilibrium within a week. It is concluded from these results that tryptophane is a human dietary essential.

Four male subjects given a diet deficient in lysine promptly developed negative N balances which persisted throughout the experimental period. A female subject on a lysine deficient diet likewise exhibited a negative N balance except for a brief period preceding the onset of menstruation, a time at which basal metabolic requirements are often decreased. The conclusion seems justified that lysine is a human dietary essential.

Three male subjects, given a diet deficient in arginine for ten days maintained their nitrogen equilibrium throughout this period. The seminal plasma of these subjects on the ninth day of the experiment revealed a reduction in the number of spermatozoa to approximately one-tenth of the normal value. A similar diet containing arginine which followed the deficiency period caused a prompt return of spermatozoa; several weeks on a normal diet, however, were required before the normal values were entirely regained. In spite of the normal N balance for ten days it seems necessary to conclude that arginine is a human dietary essential. It would appear that a temporary deficiency of arginine in the diet can be met by atrophy of spermatogenic tissue, spermatozoa being exceedingly rich in arginine.

A simple method for the laboratory diagnosis of subclinical deficiencies of thiamin, riboflavin and nicotinic acid (Demonstration). L. Emmett Holt, Jr. and Victor A. Najjar. *School of Medicine, The Johns Hopkins Univ., Baltimore, Md.* In studying these deficiencies the excretion of vitamins and certain vitamin derivatives in the urine has been utilized. The stores of thiamin and riboflavin are known to influence the excretion of these vitamins in the urine and we have shown that the urinary excretion of the fluorescent substance F_2 is dependent on available nicotinic acid.

In following the excretion of these factors several procedures have been employed: 1, *the 24-hour urinary excretion* is unsatisfactory (influenced by the immediate diet). 2, *An oral "load" test* in which excretion is followed for a period following oral administration of vitamin is not satisfactory (variations in absorption). 3, *A load test with parenterally administered vitamin* is useful but subject to certain errors. Renal insufficiency, when present, may modify the result of the test. The presence of deficiencies of other members of the B group also introduces complications. We have recently employed 4, *a "morning hour" excretion test* to obviate these difficulties. Urine is collected for a period representing one hour's excretion following an overnight fast of 12 hours. This specimen is analyzed for thiamin, riboflavin and F_2 and the value so obtained appears to depend on the body stores of thiamin, riboflavin and nicotinic acid. When the excretion of these factors in the one-hour specimen falls to zero it seems likely that the borderline of deficiency has been reached. This test permits the accurate evaluation only of mild degrees of deficiency.

A study of the first stages of casein hydrolysis by chymotrypsin and trypsin. M. K. Horwitt. *Biochemical Research Laby., Elgin State Hospital, Elgin, Ill.* If 1 mgm. of crystallized chymotrypsin is added to 10 ml. of a solution of 6 per cent casein (pH 7.5) which had been warmed to 50°C., the solution will become opaque in about 1 minute. The precipitate formed is evenly suspended throughout the solution, is soluble at 30°C. and insoluble at 50°C. The reaction is dependent upon the presence of traces of calcium which serves to precipitate the initial product of casein hydrolysis, probably paracasein.

Trypsin has a similar effect but at a greatly diminished rate. Since about 6 times as much amino nitrogen is liberated by trypsin as by chymotrypsin before the casein solution attains a chosen standard turbidity, it is possible to approximate the relative amount of proteolysis due to the components of a mixture of chymotrypsin and trypsin. Pancreatic trypsin-inhibitor markedly inhibits this chymotryptic effect and can completely inhibit the tryptic reaction.

Simple tests which may be used to distinguish between these two proteases depend upon the fact that chymotrypsin is not precipitated at pH 7.3 by heparin or insulin, whereas, trypsin is precipitated by both of these compounds. Heparin will rapidly inactivate trypsin at 25°C. but will not effect chymotrypsin.

Vitamin E and muscle degeneration in the hamster. O. Boyd Houchin. *Biochemical Laby., State Univ. of Iowa, Iowa City.* The hamster is very suitable for studying muscular changes in vitamin E deficiency. Weanling hamsters placed on a vitamin E deficient (rat) diet high in fat, showed 75 per cent mortality within three weeks, and on the rabbit diet, high in cellulose and low in fat, 100 per cent within two weeks. Death occurred suddenly without appreciable weight loss, with symptoms of muscular weakness, irritability, purulent exudate from eyes, and sometimes dystrophic paralysis. Therapeutic doses of 1 mg. of α-tocopherol produced rapid recovery, and animals remained normal for 20 days.

An average Q_{O_2} of 1.70 was found for semitendinosus muscle of normal and control hamsters (near that of rabbit muscle, slightly lower than rat muscle). The average chloride content was 59.8 mgm. per cent. The Q_{O_2} of degenerated muscle was as high as 4.55 (higher in muscle from

animals on low fat diet than on high fat diet; averages, 4.22 and 3.38, respectively). Muscle chlorides were 96.0 and 90.8, respectively, and were as high as 183 mgm. per cent in severely dystrophic muscle. After longer deprivation the Q_{O_2} values were 21 per cent lower than for younger critically deficient animals and the chloride content was also lower. This may be explained by the presence of less functional tissue.

After therapeutic doses of α-tocopherol the Q_{O_2} values approached normal values, averaging 1.73, within 30 hours. The chloride content was still slightly above normal. Some animals required a longer time, probably depending on the state of degeneration at the time of feeding. [*Aided by a grant from Parke, Davis and Company.*]

Partial synthesis of β-hyodesoxycholic acid and a procedure for the isolation of this bile acid from bile. J. LOGAN IRVIN. *Dept. of Physiological Chemistry, The Johns Hopkins School of Medicine, Baltimore, Md.* The isolation of β-hyodesoxycholic acid (β-3-α-6-dihydroxycholanic acid) from hog bile, reported by Kimura, is noteworthy because this bile acid has a hydroxyl at carbon 3 with a steric relationship identical with that of cholesterol (*cis* to the methyl group, position 10) in contrast to the configuration (*trans*) of the common natural bile acids. For the partial synthesis of this bile acid, α-hyodesoxycholic acid (α-3-α-6-dihydroxycholanic acid) was acetylated, and the diacetylhyodesoxycholic acid was preferentially hydrolyzed at room temperature in dilute methanolic potassium hydroxide. The α-3-hydroxy-α-6-acetoxycholanic acid was oxidized at room temperature with chromic oxide in 90 per cent acetic acid to 3-keto-α-6-acetoxycholanic acid. The latter was reduced at 26° under one atmosphere of hydrogen in an acid medium with platinum oxide as catalyst. Hydrogenation in an acid medium favors formation of the β-isomer, but some of the α-form was produced. Following esterification with methanol, the β-isomer was separated by digitonin precipitation. The digitonide was decomposed with pyridine and ether, and β-hyodesoxycholic acid was isolated after hydrolysis of the esters. The over-all yield was 26 per cent. After recrystallization from ethyl acetate, the compound melted at 190°. A sample of the β-hyodesoxycholic acid was oxidized to 3,6-diketocholanic acid (m.p., 164°) which was identical with a diketo acid produced by oxidation of α-hyodesoxycholic acid. The relative insolubility of the digitonide of the methyl ester of β-hyodesoxycholic acid was used as the basis for the separation of this bile acid from α-hyodesoxycholic acid during isolation from hog bile.

Criteria for differentiating vagal from non-vagal stomach pouches. EDWARD E. JEMERIN, FRANKLIN HOLLANDER and VERNON A. WEINSTEIN. *Labs. of The Mount Sinai Hospital, New York City.* In 1938 we demonstrated that the extent of vagal innervation of Pavlov pouches is less than is generally believed, because of a misconception concerning the distribution of the vagus to the dog's stomach. This led us to devise a gastric pouch with vagus supply intact, and to study the correlation of quantitative variations in secretory response with differences in vagality. Preliminary experiments with methods generally used for distinguishing vagal from non-vagal pouches (Heidenhain, Pavlov, and our own type of pouch) yielded results more irregular than would be expected from nerve destruction during the operation or subsequent degeneration. This suggested that these methods themselves might be inadequate. Accordingly, 6 definitive experimental criteria of vagality were investigated systematically, to determine whether even a qualitative differentiation could be made on this basis. Sham feeding was excluded because it is impractical as a test procedure in dogs designed for other purposes. The responses to any one criterion were inconstant, not only in different animals but in different experiments on the same animal (a total of 97 experiments were performed upon 13 dogs); also, any one pouch might be vagal by one criterion and non-vagal by another. Hence, none of these criteria are reliable for their primary purpose. Such variability results from the dependence of the criteria upon variable psychic factors (both stimulating and inhibiting), and indicates the need for a suitable chemical test stimulus. [*This investigation was supported in part by a grant from the Friedsam Foundation.*]

An electrophoretic study of concentrated cerebrospinal fluid. ELVIN A. KABAT, HAROLD LANDOW and DAN H. MOORE. *Neurological Inst., The Depts. of Neurology and Anatomy, and the Electrophoresis Laby., College of Physicians and Surgeons, Columbia Univ., New York City.* Cerebrospinal fluids of normal and elevated total protein content obtained from patients with neurological disorders were concentrated to small volume by dialysis in the cold under pressure and studied in the Tiselius electrophoresis apparatus using a micro cell of about 2 ml. capacity. The electrophoretic patterns are very similar to those of the plasma proteins. In cases where the total spinal fluid protein is elevated all of the plasma proteins are found; in those with normal protein levels, α globulin and fibrinogen are frequently not detected. The ratio of albumin to globulin is somewhat higher than that found in serum; where the total protein is increased the greater part of this increase is generally due to albumin. Alterations in the pattern of the serum proteins are reflected in the cerebrospinal fluid. Patients with lymphopathia venereum who show a high gamma globulin

in serum, have a high gamma component in spinal fluid. The A/G ratio, however, is not as low in the spinal fluid of these cases as it is in the blood. Spinal fluids with abnormal colloidal gold curves show a high gamma globulin, and separation studies indicate that all of the colloidal gold activity is contained in the gamma fraction. The albumin component tends to mask the colloidal gold reaction. The relation of the colloidal gold activity to the gamma component of spinal fluid and serum are considered in detail. [*Aided in part by a grant from the William J. Matheson Commission.*]

Myokinase and adenylpyrophosphatase. H. M. KALCKAR. *Dept. of Pharmacology, Washington Univ. School of Medicine, St. Louis.* Myokinase is an acid-stable protein (Colowick and Kalckar, J. Biol. Chem. **137**: 789, 1941) which has been found only in skeletal muscle and which is a necessary component in the hexokinase catalyzed reaction:

adenosine*di*phosphate + hexose →
adenylic acid + hexosemonophosphate

It has been found recently that myokinase plays a similar rôle in the dephosphorylation of adenosinediphosphate in muscle.

Lohmann (1936) showed that lobster muscle washed 5 or 6 times with 0.5 per cent potassium chloride splits phosphate from adinosinetriphosphate but not from adenosinediphosphate. Ljubimova and Pevsner (Biochimia (Moscow) **6**: 178, 1941) have demonstrated that myosin suspensions, when reprecipitated 2 or 3 times, maintain their ability to dephosphorylate adenosinetriphosphate (Engelhardt and Ljubimova 1939) but have lost the ability to dephosphorylate adenosinediphosphate.

Addition of a small amount of myokinase restores the ability of these systems to dephosphorylate adenosinediphosphate. A myosin suspension, which in 15 minutes at 25° splits only 5 μg. of P from adenosinediphosphate, liberates 25 μg. of P in the presence of 10 μg. of myokinase per ml. The dephosphorylation of adenosinediphosphate in washed lobster muscle can be increased 8 fold by addition of myokinase.

The necessity of myokinase for the liberation of phosphate from adenosinediphosphate seems, however, to be limited to muscle tissue since liver extracts readily dephosphorylate adenosinediphosphate although such extracts are completely free of myokinase (Colowick and Kalckar. J. Biol. Chem. **140**: xxix, 1941).

A new method for the preparation of chorionic gonadotropic hormone. P. A. KATZMAN, MARCOS GODFRID, C. K. CAIN and E. A. DOISY. *Laby. of Biological Chemistry, St. Louis Univ. School of Medicine, St. Louis, Mo.* The method is based on the adsorption of the gonadotropic material of acidified pregnancy urine on a column of permutit and its elution with ammonium acetate or hydroxide in aqueous ethanol. The adsorption is complete even after passing 565 liters of urine through 2 kgm. of permutit. In most instances, the elution removes more than 90 per cent of the adsorbed activity, the bulk of which is usually sharply confined to one or two fractions of the eluate. The columns can be used repeatedly after washing with dilute ammonium hydroxide and hydrochloric acid to regenerate the permutit.

The active material is fractionally precipitated from the ammonium acetate-aqueous alcohol solution by means of ethanol. The fractions precipitated between 70 and 80 per cent alcohol usually contain all of the activity. The potency of these preparations has ranged up to 16,000 rat units (8,000 international units) per mgm. The ammonium acetate increases the solubility of the hormone in ethanol without contaminating the precipitates since it is soluble in this solvent. A substantial purification of the fractions ranging in activity from 1,000 to 10,000 rat units per mgm. can be accomplished by extracting the dry powder with solutions of ammonium acetate in aqueous ethanol and precipitation by the addition of the alcohol. The entire procedure involves very little loss of activity. A study of the chemistry of this purified product is now in progress.

Quantitative studies of the activity of the hormones of the adrenal cortex. EDWARD C. KENDALL. *Division of Biochemistry, Mayo Foundation, Rochester, Minn.* Qualitative differences in the physiologic effects of the hormones of the adrenal cortex have been established. There are also quantitative differences in the activity of the crystalline hormones when they are compared with the activity of the whole, unfractionated extract. The effect of the extract on carbohydrate metabolism is due to steroids with an oxygen on C_{11}. But these compounds are present in the extract in a form which produces an enhancement of their physiologic activity. The enhanced activity is not destroyed by heating a water solution to 80° for one hour. Exposure to vigorous aeration at pH 7.5 in water or benzene does not affect the activity but extraction of the hormones with water at room temperature from benzene results in serious loss of activity. The structure of the steroid compounds remains unchanged and subsequently they may be separated in crystalline form.

The effect of the unfractionated extract on renal function is also enhanced and can be destroyed by repeated extraction of a solution in benzene with water. The loss of activity is not produced by hydrolysis, oxidation or reversible dissociation of the essential components. The enhanced activity of the whole, unfractionated extract is of the order

of 10 to 20 times that of the hormones after separation in crystalline form. The instability of the enhanced form of the hormones emphasizes the necessity of the use of a method of preparation which will not only insure complete extraction from the gland but which will also safeguard the enhanced activity.

Effect of riboflavin on the metabolism of glucose and fructose by lactobacillus helveticus. J. RAYMOND KLEIN and HENRY KAMIN. *Dept. of Biochemistry, Duke Univ. School of Medicine, Durham, N. C.* Preparations of *Lactobacillus helveticus* obtained from cultures in which growth was not limited by the concentration of riboflavin oxidize glucose and fructose readily. Preparations of the organism obtained from cultures in which growth was limited by the concentration of riboflavin oxidize the sugars slowly. The ability of the latter preparations to oxidize the sugars can be increased by the addition of riboflavin. This increase in the ability of the preparations to oxidize the sugars is accompanied by an increase in the concentration of flavin-adenine dinucleotide. The presence of the dinucleotide was indicated by the ability of heated preparations of the organism to form the d-amino acid oxidase when mixed with the specific protein of the oxidase. The increase in oxidizing ability can also be obtained by the addition of flavin-adenine dinucleotide, but not by the addition of lumiflavin. In the oxidation of the sugars oxygen is used and carbon dioxide produced. Hydrogen peroxide is produced during the reaction as indicated by the conversion of added hemoglobin to methemoglobin. The rate and extent of acid production (lactic and unidentified acids) by preparations of the organism are not influenced by addition of riboflavin and does not depend upon the concentration of riboflavin in the culture medium.

Ketone metabolism in the mouse. ALFRED E. KOEHLER and EMANUEL WINDSOR. *The Sansum Clinic and the Santa Barbara Cottage Hospital, Santa, Barbara, Calif.* Mice could be analyzed readily for the various ketones, including the volatile ones, by maceration in ice water to a thin creamy fluid in a closed system by high speed revolving knives.

After intraperitoneal injection of 75 mg. of acetone in saline per 100 grams body weight, 55 per cent was destroyed in 4 hours after correcting for respiratory and urinary loss. This represents a utilization of about 10 mgm. per hour per 100 gram. Only 2.6 per cent of the acetone destroyed could be accounted for as extra acetoacetic and β-hydroxybutyric acid in the tissues at the end of the 4 hour period.

After intraperitoneal injection of 150 mgm. of acetoacetic acid per 100 grams, 92.8 per cent was destroyed in 2 hours, representing 65.5 mgm. per hour per 100 grams body weight. Only the equivalent of 4.3 per cent of the acetoacetic acid destroyed was recovered as acetone and β-hydroxybutyric acid at the end of the 2 hour period. The rate of absorption of acetone and acetoacetate from the peritoneal cavity was found to be sufficiently rapid so as not to be a factor in our experiments.

Compared to experiments on man (Koehler, Windsor and Hill, J. Biol. Chem. **140:** 811, 1941), the utilization of acetone in the mouse is very much greater. The utilization of acetoacetic acid in man was found to be approximately 3.4 mgm. per hour per 100 grams. In the mouse the value is about 19 times as great.

The response of the cinchonas and other bases to the photelmetric estimation of quinine. GRANVIL C. KYKER. *Dept. of Biological Chemistry, School of Medicine, Univ. of North Carolina, Chapel Hill.* The recently reported method for the photelmetric estimation of quinine is not specific but responds generally to nitrogenous substances which, according to the procedure: *a*, are extractable from biological samples, and *b*, yield turbidities with silicotungstic acid. This study began with the latter so that the degree of extraction could be interpreted.

All measurements were based on a stock solution of each alkaloid containing 1000 mgm. per liter in 0.03 N hydrochloric acid. Consecutive half-dilution of each stock was continued so long as the last dilution gave a recognizable turbidity with silicotungstic acid. Specific rotations were observed on all dilutions of sufficient concentration. A portion of each stock was precipitated with an excess of silicotungstic acid and the composition of the precipitate was determined by ignition and weighing the residual silicon and tungsten oxides.

With each alkaloid various samples of different commercial origin were used. Studies on four quinine, six quinidine, two hydroquinidine, and two cinchonine samples were completed. The four quinine samples responded similarly. Hydroquinidine and cinchonine gave turbidities similar in appearance to that from quinine but less dense than the same concentration of quinine. Quinidine gave a very unstable turbidity which gave readings comparable to that from one-sixth its concentration of quinine. All determinations of specific rotation and composition of the silicotungstates confirm the purity of most of the samples. A comparison of various other cinchona derivatives and also non-alkaloidal bases is in progress.

A new method for the estimation of methionine. THEODORE F. LAVINE. *Lankenau Hospital Research Inst., Philadelphia, Pa.* Methionine by virtue of its thioether structure is capable of

reacting with iodine to form a diiodide, $RSCH_3.I_2$, or more properly an iodo sulfonium iodide, $[RS(I)CH_3]^+I^-$. This compound is, under suitable conditions, quantitatively regenerated into its components, although in time it is transformed into methionine sulfoxide. If the regeneration of iodine from the diiodide were a unique characteristic of this product of protein hydrolysis, among those that consume iodine, it would furnish the basis of a rapid method for the estimation of methionine. In testing this, the general procedure has been to add an excess of iodine to the methionine in 0.5 to 1M KI solution, they destroy the excess exactly by thiosulfate, make the solution acid, and titrate the liberated iodine with thiosulfate. With solutions of methionine only, precise results were obtained at pH 7, 8 and 9. The rate of formation of the diiodide increases as the alkalinity increases. Cystine is also capable (to a small extent) of forming a diiodide but this is overcome by using less than a 5 per cent excess of iodine and by using 1 M KI.

In order to test for the possible interference of other amino acids, a casein hydrolysate was tested and found to yield 2.8 per cent of methionine at pH 7, 8 and 9. However, hydrolysates of other proteins yielded higher values at pH 9 than at pH 8 or 7, suggesting interference from other amino acid than cystine. This point is being investigated.

The blood pressure of the Eskimo. VICTOR E. LEVINE. *Dept. of Biological Chemistry and Nutrition, Creighton Univ. School of Medicine, Omaha, Neb.* The systolic and diastolic blood pressures of Oriental peoples have been reported to be lower than those of the Caucasian race. In the course of medical and biological surveys of the Eskimos, conducted under a grant furnished by the National Institute of Health, U. S. Public Health Service, we determined the blood pressure of 823 Eskimos, ranging in age from 8 to 80 years, and living in the islands of the Bering Sea, along the Bering coast from St. Michael to Wales, and along the Arctic coast from the Bering Strait to Point Barrow. For the determination of normal blood pressure, a delimited group of Eskimos was taken with a systolic pressure of not more 129 mm. and a diastolic pressure of not more than 82 mm.

The following results were obtained: in the age group 8–12 years, the males averaged 97.9 ± 1.18 mm. systolic and 65.6 ± 0.81 mm. diastolic, the females 97.1 ± 1.23 mm. systolic and 65.7 ± 0.67 mm. diastolic. In the age group 13–15 years, the males averaged 101.2 ± 1.45 mm. systolic and 65.9 ± 0.93 mm. diastolic, and the females averaged 105.6 ± 1.5 mm. systolic and 67.5 ± 1.06 mm. diastolic. In the age group 16–20 years, the average for the males was 107.2 ± 1.22 mm. systolic and 67.6 ± 0.96, mm. diastolic, and for the females 108.4 ± 1.28 mm. systolic and 69.6 ± 1.0 mm. diastolic. In the age group 21–80, the average for the males was 109.9 ± 0.68 mm. systolic and 71.0 ± 0.44 mm. diastolic, and for the females 109.6 ± 0.72 mm. systolic and 70.6 ± 0.46 mm. diastolic. A systolic blood pressure of 90 mm. was often encountered, and this low blood pressure coexisted with a high degree of physical efficiency.

The figures indicate that the blood pressure of the Eskimos, a Mongolid type, subsisting on a high meat diet, is below that of the white race and falls within the range determined for other Mongolid groups, such as the Chinese, Japanese and Koreans.

The relation of vitamin A intake to growth and to concentration of vitamin A in the blood plasma, liver and retina. J. M. LEWIS, OSCAR BODANSKY, K. G. FALK and G. MCCUIRE. *Dept. of Pediatrics and the Dept. of Laboratories, Beth Israel Hospital, and the Laby. of Industrial Hygiene, New York City.* Different levels of vitamin A were fed daily to groups of rats 3 to 4 weeks old, for a period of 6 weeks. The rate of growth increased with increasing intake until it reached an optimum at an intake of 25 I. U. daily, and remained at this optimum (up to 1000 I. U. daily). The average plasma vitamin A concentration also increased with increasing levels of vitamin A intake, reaching an optimum at an intake of 50 I. U. daily, (and up to 1000 I. U. daily). There was no liver storage at intakes of 10 I. U. or less daily, slight storage at 25 I. U., and increasingly larger storage at higher intakes. Retinal concentrations of vitamin A were usually low at zero intake and reached an optimal value at an intake of 2. I. U. daily.

When rats were placed on a vitamin A-free diet, the plasma vitamin A concentration remained high in those with large liver stores, but fell rapidly in those with low reserves. The retinal concentration of vitamin A remained high despite the low vitamin A plasma concentration and the absence of vitamin A in the liver. The minimum vitamin A requirement in the rat was 2 I. U. daily or 20 I. U. per kgm. body weight. Optimal growth occurred at 25 I. U. daily intake, optimal blood concentrations at 50 I. U. daily, and good liver reserves at 100 I. U. daily.

Carbohydrate metabolism of animals treated with methyl testosterone and testosterone propionate. LENA A. LEWIS and E. P. MCCULLAGH. *Dept. of Biochemistry, Cleveland Clinic Foundation, Cleveland, O.* The use of methyl testosterone in patients is followed by a decrease in tolerance for glucose (McCullagh and Jones). The studies have been extended to rabbits. Normal oral and intravenous glucose tolerance tests were made on adult male rabbits maintained on a constant diet, after which

the animals were treated with 5, 10 or 20 mgm. of methyl testosterone propionate per day. After 20 to 22 days of treatment with methyl testosterone a greatly decreased glucose tolerance was observed (both intravenous and oral tests). Following a similar period of testosterone propionate treatment, no significant deviation from the normal was found. When no further modification in the sugar tolerance of the methyl testosterone treated rabbits was observed (39 to 41 days) liver and muscle glycogens were determined. The glycogens of the testosterone propionate treated rabbits were determined after an equal or longer period of treatment (41 to 55 days). The liver glycogens of the methyl testosterone treated animals were markedly below those of the normal controls. The testosterone propionate treated animals, however, showed a trend toward a slightly increased liver glycogen level. No significant change was observed in the muscle glycogen values of either group.

The fact that there is a marked increase in the basal metabolic rate of the methyl testerone treated animals suggested that the hypermetabolism itself might be affecting the glucose tolerance in a manner similar to that operative in hyperthyroidism. The glucose tolerance of thyroidectomized rabbits before and after treatment with methyl testosterone is being determined.

A substance found in glycogen preparations and biotin concentrates which affects respiration. B. LIBET and K. A. C. ELLIOTT. *Inst. of the Pennsylvania Hospital, Philadelphia.* It was previously reported by us (J. Biol. Chem. **136**: 797, 1940) that the addition of glycogen initially stimulated and later inhibited the respiration of brain suspensions. These results were obtained with commercial glycogen (c. p. Pfanstiehl) from liver and purified glycogen from scallop muscle. It has been found that the effects were due to an impurity present in many glycogen preparations. The active substance can be partially dialysed out of glycogen solutions. Glycogen can be freed of the active substance by precipitating the glycogen with alcohol from N acid solution. A crude preparation of the active substance, free of glycogen, consists in the precipitate obtained on bringing a concentrated hot water extract of liver to pH 2.5.

Active preparations have been obtained from livers of various animals, scallop muscle, and yeast. "Injectable biotin concentrate" (S. M. A.) is strongly active. The following substances do not behave as the active substance in question: biotin, folic acid, choline, p-aminobenzoic acid, glutathione, cozymase, adenylic acid, xanthine, hypoxanthine, uric acid, alanine, citrate, succinate, fumarate, malate, pyruvate, lactate. The activity of the substance is destroyed by ashing but not by concentrated HCl nor by heating with 50 per cent NaOH.

Cytolysed brain suspension can be centrifuged to give a fluid the respiration of which is negligible but is markedly stimulated for a short time by the substance. Liver suspensions are also stimulated but the later inhibition does not occur. The extra oxygen uptake due to the substance is accompanied by little or no CO_2 evolution.

Isolation of acetyl phosphate as precursor of acetic acid in pyruvate oxidation. FRITZ LIPMANN. *Surgical Research Labs., Harvard Medical School, Boston, Mass.* The earlier observed dependence of pyruvic acid oxidation by Bacterium Delbrückii on the presence of inorganic phosphate led to the search for a phosphorylated intermediate. Lately the accumulation of an extremely unstable phosphate derivative could be demonstrated (J. Biol. Chem. **134**: 463, 1940). Our suspicion that this is the mixed anhydride of acetic acid and phosphoric acid has been confirmed now through the isolation of the compound.

Dried bacteria were incubated with appropriate amounts of pyruvate, phosphate and fluoride until the larger part of the inorganic phosphate had disappeared. The protein and part of the inorganic phosphate were removed with alcohol. The remaining inorganic phosphate was precipitated as calcium phosphate. The slightly soluble silver salt of the unstable phosphate derivative was then precipitated with excess silver nitrate. Several reprecipitations finally yielded a preparation of the composition of di-silver mono-acetyl phosphate: Found: Ag, 63.0, P, 8.5, and acetic acid, 17.4 per cent. Calculated: Ag, 63.6, P, 9.1, and acetic acid, 17.7 per cent.

Glyceride structure of rat body fats. HERBERT E. LONGENECKER and KARL F. MATTIL. *Dept. of Chemistry, Univ. of Pittsburgh, Pa.* An examination has been made of the glyceride structures in rat body fats formed during the ingestion of large amounts of saturated acids (lauric, palmitic, and stearic) fed as their triglycerides. Under these conditions body fats were found with unusually high proportions (45 to 65 per cent) of saturated acids. The glyceride distribution of these body fats will be discussed in terms of a general theory, based upon the complete random distribution of all available acids, for the formation of animal body fats.

The effect of "Sulfa" drugs on the thyroid gland in rats and mice. JULIA B. MACKENZIE and C. G. MACKENZIE. *Dept. of Biochemistry, School of Hygiene and Public Health, The Johns Hopkins Univ., Baltimore, Md.* The addition of 1 or 2 per cent of sulfaguanidine to a purified diet or the McCollum stock diet produces thyroid enlargement in rats, characterized initially by a transformation of the cuboidal epithelium to the

columnar type, and a great decrease in colloid. These changes occur in both immature and adult rats, and have been observed within 4 weeks on the 1 per cent level and in 1 to 2 weeks on the 2 per cent level of the drug. The basal metabolic rate of rats fed sulfaguanidine and the results of attempts at prophylaxis with iodide and vitamin C will be reported.

One and a half per cent of sulfanilic acid and 0.55 per cent of guanidine carbonate, alone or in combination, did not produce microscopic changes or thyroid enlargement in 4 weeks. Gross and microscopic changes resembling those effected by sulfaguanidine were produced by feeding 0.66 per cent of thiourea for 3 weeks.

Sulfadiazine, sulfapyridine and sulfanilamide, when fed at 1 to 2 per cent levels for 4 weeks, caused enlargement and hyperemia of the rat thyroid. The thyroids of mice fed sulfaguanidine, sulfapyridine and sulfathiazole at 2 to 3 per cent levels for 3 weeks weighed approximately 3 times more than the thyroids of control animals. The microscopic picture of the thyroid glands of rats and mice fed these and other "sulfa" drugs will be described.

Lipids of the fasting mouse. II. The fat-to-water relation in the liver and the fractionation of the liver phospholipids. P. L. MacLachlan, Harold Carpenter Hodge, W. R. Bloor, Eileen A. Welch, Frederick L. Truax and J. D. Taylor. *Dept. of Biochemistry and Pharmacology, School of Medicine and Dentistry, Univ. of Rochester, Rochester, N. Y.* In fasting, the neutral fat which enters the liver during the first two fasting days does so without disturbing the liver-water relationships other than by diluting the liver substance. The weight of phospholipid decreases by half during the four days fast but the phospholipid concentration (weight per cent) and the lecithin-to-cephalin ratio are not appreciably altered. Eighty per cent of the α lecithin and β cephalin originally present disappears in four days' fast. In contrast, the amounts of β lecithin and α cephalin remain nearly constant.

Serum iodine fractions. E. B. Man, E. F. Gildea and A. E. Smirnow. *Dept. of Psychiatry, Yale Univ. School of Medicine, New Haven, Conn.* The presence of protein-bound and inorganic iodine in blood has been demonstrated by a number of investigators. Previously reported experiments from this laboratory indicated that most of the iodine was contained in serum in the organic form. Methods for differentiating these iodine fractions such as precipitation with organic solvents, dialysis and precipitation of proteins with heat and acetic acid were inaccurate or lengthy.

A simple method (Trevorrow) has been used for the separation of iodine fractions in 6 cc. aliquots of serum. Serum is precipitated with the Somogyi reagents, zinc sulfate and sodium hydroxide. After 8 washings iodine is determined in precipitate and filter paper. With this method little inorganic iodine has been found in the serum of normal subjects.

Administration to 5 euthyroid subjects of sufficient Lugol's to elevate total iodine to between 50 and 522 gamma percent did not change the precipitable iodine from normal levels of 6 gamma percent. After treatment was stopped there was no decrease in precipitable iodine, as total iodine decreased.

Seven hyperthyroid patients had high total or precipitable iodine values. When hyperthyroid symptoms were relieved by iodine therapy, precipitable iodine diminished to between 9.6 and 5.9 gammas per cent although total iodine remained high.

It is concluded that this zinc sulfate precipitation method differentiates protein-bound from inorganic iodine in serum, and that the organic fraction contains most of the normally-occurring iodine in blood. This fraction is increased in hyperthyroidism and decreases toward normal during iodine therapy although the total iodine is markedly elevated.

Iodine metabolism in the thyroid gland. Walter Mann, Charles Lebond and Stafford Warren. *Dept. of Radiology, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.* By injecting radioactive iodine without "carrier" into dogs and comparing the proportion of radioactivity found in the various thyroid gland iodine fractions, it has been possible to demonstrate *a*, that diiodotyrosine is the natural precursor of thyroxine; *b*, that inorganic iodine does exist in the normal non-iodized thyroid gland; *c*, that iodination of tyrosine apparently does not take place inside of the thyroid cell, and *d*, that 1.6 per cent of the total thyroxine contained in the thyroid gland is formed per hour.

Determination of thiamine in urine by the thiochrome method. Estimation of the blank. Harold L. Mason and Ray D. Williams. *Division of Biochemistry, Mayo Foundation, Rochester, Minn.* It has been shown (Mason and Williams, J. Biol. Chem. **140**: 417, 1941) that the usual blank determination in the method of Hennessy and Cerecedo (J. Am. Chem. Soc. **61**: 179, 1939) is not applicable to urine since the value so obtained may at times be greater than the apparent value for thiamine. It has been found that complete destruction of thiamine up to 6 micrograms in 10 cc. of urine can be accomplished by heating with 25 mgm. of sodium sulfite at pH 5 for fifteen minutes at 100°. When the treated urine is processed as usual the resulting fluorescence gives a value for the amount of non-thiamine material which is measured as

thiamine in the usual determination. This blank value must be determined on the same volume as the total thiamine since it does not increase in proportion to the volume. Normal urine and that of subjects on a diet greatly restricted in thiamine contain from 1 to 5 micrograms of non-thiamine material when calculated as thiamine. Only when the excretion of thiamine is very small, or when doses of 150 mgm. or more of nicotinic acid are being given, is the blank value of significance. In the latter instance the relatively large amounts of metabolic products of nicotinic acid may interfere to such an extent that the direct value obtained for thiamine is greatly in error. Determination of the blank after destruction of thiamine by sulfite permits correction for this interference.

Observations upon the toxicity of blue-green algae. M. F. MASON and R. E. WHEELER. *Depts. of Biochemistry and Preventive Medicine, Vanderbilt Univ., Nashville, Tenn.* The known toxicity of the blue-green algae, *microcystis aeruginosa*, has been studied with mice, rats, guinea pigs and cats as test objects. Fresh, concentrated algal suspension was administered subcutaneously or intraperitoneally, or partially purified extracts containing the toxic agent were injected by these routes or intravenously.

Following subcutaneous or intraperitoneal administration of a lethal dose of partially purified extract (equivalent to 3 mgm. of algae, dry weight, for mice or approximately 1 gram for a 1500 gram cat) there is a latent period (20 min., mice; 2-3 hrs., cats) followed by pallor, fall in blood pressure, tachycardia, decline of body temperature, hyperglycemia, and terminally a marked reduction in hematocrit reading, hemoglobin, red count, and total serum protein. Death follows respiratory failure. Autopsy reveals a markedly congested liver, dilatation of the right heart, but little evidence of general venous congestion. Chromodacryorrhea was not observed.

The toxic agent had the following effects upon isolated tissues. The perfused frog's heart was thrown into systolic arrest by minute amounts. The reaction of virgin guinea pig uterus was equivocal. The over-all oxygen consumptions of isolated slices of rat kidney, liver, and diaphragm and brain brei in Ringer-phosphate-glucose (Barcroft-Warburg technique) were not affected by concentrations lethal to the intact animal.

Partially purified preparations of the toxic agent were inactivated by heating with acid and alkali. Acid hydrolysis was accompanied by an increase of reducing substances, but this "sugar" might have arisen from contaminating material.

Blood, blood chemical and various physiological studies, and attempts at purification are in progress.

Chemical studies on the bone marrow related to hematopoietic activity. R. H. MCCOY and M. O. SCHULTZE. *Dept. of Chemistry, Univ. of Pittsburgh, Pa.* Simultaneous analyses for many constituents should reveal correlations between chemical composition, hematopoietic activity and cytology of the bone marrow. Analytical comparisons of the bone marrow from anemic iron-deficient and anemic copper-deficient rats with that from normal rats and from rats recovering from copper deficiency are reported.

The fat content of the marrow was variable, depending upon the age and the state of anemia of the rats. This necessitates expression of results on the basis of fat free solids.

In the marrow of normal rats and of rats recovering rapidly from anemia, the amount of hemoglobin present was relatively small. Therefore, during rapid hematopoiesis the rate at which hemoglobin was synthesized and entered the bloodstream was very fast. Under those conditions there was also a rapid turnover of nitrogenous compounds. In the marrow of copper-deficient rats, a larger proportion (about 68 per cent) of the total fat-free solids was water soluble than in normal marrow (about 48 per cent). The nitrogen content of this fraction remained unchanged, however.

From 10 to 15 per cent of the total nitrogen of the marrow existed as N.P.N. In severe anemia this fraction was increased, but returned to normal during rapid recovery. Analyses show that compounds other than ammonia, urea, amides, or free amino acids contributed largely to this fraction. [*This work was aided by grants from the John and Mary R. Markle Foundation, New York, and from the Buhl Foundation, Pittsburgh.*]

Effects of inositol upon liver fat in the rat. E. W. MCHENRY and GERTRUDE GAVIN. *Dept. of Nutrition, School of Hygiene, Univ. of Toronto, Canada.* Several types of fatty livers may be produced in the rat by dietary means. The thiamin type, caused by supplying thiamin with a low-choline diet, is prevented by small amounts of choline. Cholesterol fatty livers, produced by feeding cholesterol, are partially prevented by the administration of choline, which prevents an increase of neutral glycerides but not of cholesteryl ester. Biotin fatty livers, caused by giving biotin, are characterized by containing large amounts of cholesterol and are not prevented by choline. Inositol has no effect upon thiamin fatty livers, exerts an additive effect with choline on cholesterol fatty livers, and prevents the biotin type of fatty liver. The simultaneous administration of other known B vitamins with thiamin produces fatty livers which are not completely prevented by choline; under these circumstances inositol has an additive effect with choline. Inositol prevents the marked accumulation of

cholesterol in the liver which occurs when biotin is given.

Precipitation of proteins by organic sulfates and sulfonates. THOMAS L. MCMEEKIN. *Eastern Regional Research Laby., Bureau of Agricultural Chemistry and Engineering, U. S. Dept. of Agriculture, Philadelphia, Pa.* The relative effectiveness of compounds of several homologous series, such as sulfate esters of primary alcohols, sulfonates of diesters of succinic acid, and sulfonates of benzene ring compounds, in precipitating proteins has been determined. For these studies, solutions of the albumin fraction of milk, crystalline lactoglobulin, and crystalline egg albumin were used. The amount of nitrogen precipitated under constant conditions of temperature, acidity, and concentration by each precipitating substance was determined. Under these conditions it was found that sulfates and sulfonates were ineffective precipitating agents when the substances contained less than eight CH_2 groups. However, when the number of CH_2 groups was increased beyond eight, the precipitating effectiveness was increased with each additional CH_2 group until a maximum amount of precipitation per mole of reagent was reached. The further addition of CH_2 groups to the molecule was without effect. The straight carbon-chain compounds were more effective than the corresponding compounds with branched carbon chains of the same carbon content. The sulfonates of benzene ring compounds were less effective in precipitating protein than the corresponding straight-chain compound, but resembled the compounds with two branch chains, such as the sulfonates of diesters of succinic acid. The sulfates and sulfonates of compounds containing large amounts of carbon were excellent protein precipitants under a variety of conditions.

Possible steps in the formation of taurine from cystine. GRACE MEDES and NORMAN F. FLOYD. *Lankenau Hospital Research Inst., Philadelphia, Pa.* The insoluble protein remaining when liver brei was extracted with phosphate buffer at pH 6.7 contained enzymes capable of simultaneously oxidizing and decarboxylating cystine. Both reactions occurred more slowly than the oxidation of cysteine to the sulfonic acid, which may also be performed by this same enzyme preparation. Partial purification will be described.

Various substrates have been employed with the oxidizing and decarboxylating enzymes, and the degree of specificity and probable course of the reaction have been postulated. If decarboxylation occurred first, oxidation could not take place, as cystamine is inert. If complete oxidation of the sulfur was first accomplished, decarboxylation failed to occur, since cysteic acid does not yield carbon dioxide. Therefore, it is suggested that a preliminary partial oxidation of the sulfur must occur, following which, decarboxylation must take place with subsequent complete oxidation of the sulfur. In line with this postulation, it was found that oxidation of the disulfoxide of cystamine took place with great ease.

Three enzymes, then, would seem to be concerned in this series of reactions in which taurine may be produced from cystine: one which oxidizes cystine to the disulfoxide, one which decarboxylates cystine disulfoxide, and another oxidase which brings about the further oxidation of the sulfur atom.

Muscle proteins of the rat and of the octopus. JOHN W. MEHL and EDWIN L. SEXTON. *Dept. of Biochemistry, School of Medicine, and the Labs. of the Hancock Foundation, Univ. of Southern California, Los Angeles.* The soluble proteins of striated muscle from the rat have been studied by the method of electrophoresis. The electrophoresis patterns indicated the presence of at least four proteins in addition to myosin. While these have not been definitely identified in terms of globulin X, the myogens, and myoalbumin, it appears that there is at least one globulin which has not previously been reported.

The electrophoresis patterns of the extracts of the smooth muscle of the octopus showed two well defined peaks in addition to that which was probably due to the myosin. However, the presence of other components was definitely indicated by the shape of these patterns.

Studies on an inherited bleeding disease in swine. E. T. MERTZ. *Dept. of Agricultural Chemistry, Univ. of Missouri, Columbia.* In a search for differences between normal swine and swine afflicted with an inherited bleeding disease, we found that the affected animals had prolonged bleeding times when tested by Doettl and Ripke's method of bleeding into isotonic saline solution. For a suitable evaluation of this and other tests, three severely affected animals and five normal controls were examined at weekly intervals for a period of three months. The average results on the bleeders showed a 6-fold prolongation of the liquid bleeding time, a normal Duke's bleeding time, and a 2-fold prolongation of the whole blood coagulation time. The nature of the defect is discussed. [*Aided by a grant from the John and Mary R. Markle Foundation.*]

The colorimetric determination of uronic acids. KARL MEYER, HENRY S. BLOCH and ELEANOR CHAFFEE. *Dept. of Ophthalmology, College of Physicians and Surgeons, Columbia Univ., New York City.* The micro-determination of uronic acids, especially of hyaluronic acid in aqueous humor and other natural fluids, has interested our laboratory for several years. Investigations of Tollens' naphthoresorcinol test, the most prom-

ising for this work, led to the following conclusions: 1. None of the methods available gave accurate results for uronic acids in native fluids, with the possible exception of normal urine, since carbohydrates and other compounds containing carbonyl groups as well as proteins and their decomposition products interfered. 2. A simple method was found for the accurate micro-determination of *pure* uronides, both free and conjugated. 3. Conjugated uronides had to be hydrolyzed, methanolysis being the method of choice; acid hydrolysis tended to decarboxylate the uronic acid. 4. A colorless aqueous solution of highly purified naphthoresorcinol proved of great importance for quantitative results. 5. The color intensity of the uronic acid naphthoresorcinol condensate was increased by $K_2S_2O_8$. 6. Ether was unsuitable as an extractant for the condensate. Benzol was the extractant of choice, as reported previously by other workers. 7. A mechanism of the reaction between uronic acids and naphthoresorcinol is suggested, whereby two moles of naphthoresorcinol condense with one mole of uronic acid giving a xanthene derivative, the color of which is due to oxidation to a quinoid structure.

Action of synthetic detergents on protein. ZELMA BAKER MILLER, HENRY M. LEMON and BENJAMIN F. MILLER. *Walter G. Zoller Memorial Dental Clinic and the Dept. of Medicine, Univ. of Chicago, Ill.* The effects on bacterial metabolism and viability of cationic and anionic detergents were investigated previously, and certain generalizations were derived (Z. Baker, R. W. Harrison and B. F. Miller, J. Exper. Med. **73**: 249; **74**: 611, 1941. The inhibitory action of synthetic detergents on bacteria may be prevented by previous or simultaneous addition of phospholipids (Z. Baker, R. W. Harrison and B. F. Miller, J. Exper. Med. **74**: 621, 1941). To determine the extent to which the anti-bacterial effects of detergents can be ascribed to protein denaturation, studies have been made with purified egg albumin. The ferricyanide and porphyrindin techniques were used to determine the degree of protein denaturation. The intensity of denaturation by detergents parallels to some degree their anti-bacterial activity. In homologous series of anionic detergents, the extent of denaturation varies with the chain length of the hydrophobic group, corresponding to the effects observed on bacteria. Phospholipids prevent the denaturation of protein by detergents.

The effect of temperature on response to anoxia. FERRIN B. MORELAND. *Biochemical Laby., State Univ. of Iowa, Iowa City.* Earlier work by Johlin and Moreland showed that turtles will usually recover from 24-hours exposure to pure nitrogen, and that this anoxia produces a decrease in blood pH and alkali reserve and an increase in sugar and lactate. Further experiments showed that this resistance was less at 37° than at 20° but was still much greater than for mammals; turtles lived about 3 hours in nitrogen at the higher temperature. Although 3 hours at 37° was somewhat harder on a turtle than 24 hours at 20°, the changes which it produced in the composition of the blood were less. The pH was affected almost as much, falling from an average normal of 7.62 to 6.87 at 37° and 6.79 at 20°. Sugar concentration rose somewhat less, from 60 mgm. per cent to 380 at 37° and 505 at 20°. Alkali reserve and lactate values showed the most difference in the effect of the two treatments. Bicarbonate values fell from a normal of 76 vol. per cent CO_2 to 42 at 37° and to 20 at 20°; lactate values rose from 22 mgm per cent to 305 and 700 respectively.

Blood, plasma and "available fluid" volumes of boys 10 to 16 years of age. MINERVA MORSE, FREDERIC W. SCHLUTZ and DONALD E. CASSELS. *Dept. of Pediatrics, Univ. of Chicago, Ill.* The blood, plasma, and "available fluid" volumes of 40 healthy boys 10 to 16 years of age have been determined by use of the dye T-1824, thiocyanate, and hematocrit readings. The nutritional status of the boys has been rated according to both McCloy and Wetzel.

Simple and multiple correlations coefficients have been calculated between blood, plasma, and "available fluid" volumes on the one hand and age, height, weight, surface, index of build, index of fat, and developmental levels on the other. With blood and plasma volumes better correlations were obtained from logarithmic functions. Simple correlations between blood, plasma, or "available fluid" volumes and body surface were as high as multiple correlations involving other factors. Blood volume and surface gave the highest correlation coefficient. With few exceptions, the following equations have been found to predict volumes to within ±10 per cent:

log blood volume (cc.) = 2.926 + 0.466 body surface ($m.^2$)
log plasma volume (cc.) = 2.749 + 0.423 body surface ($m.^2$)
"available fluid" volume (l.) = −6.78 + 13.96 body surface ($m.^2$)

Comparison of our results with those of Brines, Gibson, and Kunkel (Pediatrics **18**: 447, 1941) for younger children, suggests that at approximately ten years the blood and plasma volumes of boys increase rapidiy with reference to body height, weight, and surface, but further data will be required to determine whether this apparent spurt is real or a phenomenon of sampling. The majority of our younger boys were short for their age and slightly underweight.

Influence of solvent on the ultra violet absorp-

tion curve of vitamin A. KENNETH MORGAREIDGE. *Nopco Vitamin Labs., National Oil Products Company, Harrison, N. J.* The common use of isopropanol (99 per cent) as solvent in the spectrophotometric determination of vitamin A has become a problem, in that a grade of sufficient purity for ultra violet work is now difficult or impossible to obtain commercially. Although cyclohexane of suitable quality is available at present, the indiscriminate substitution of one solvent for another offers difficulties, as pointed out by Adamson and Evers (*Analyst* **66**: 106, 1941). It is well known that a polar and a non-polar solvent often do not give identical extinction values for the same solute. Using the Beckman quartz photoelectric spectrophotometer (wavelength scale accurately adjusted at the 3131.6 Å doublet of mercury), the adsorption curve of vitamin A from a number of sources was examined in both solvents. The following peak values for $E^{1\ \text{per cent}}_{1\ \text{cm.}}$ are typical: Vitamin A alcohol (crystals), 1783 (at 325 mμ) in isopropanol, 1620 (at 327 mμ) in cyclohexane; unsaponifiable fish oil concentrate, 777 (at 325 mμ) in isopropanol, 738 (at 328 mμ) in cyclohexane; ester concentrate (distilled), 114 (at 327 mμ) in isopropanol, 112 (at 328 mμ) in cyclohexane; ester concentrate (differential solubility method), 131 (at 326 mμ) in isopropanol, 130 (at 327 mμ) in cyclohexane; U.S.P. reference oil, 0.892 (at 323 mμ) in isopropanol, 0.856 (at 323 mμ) in cyclohexane. A series of other materials of widely varying potencies and unknown ester-alcohol ratios gave intermediate values. These data yield a number of obvious implications bearing on an exact technique for the spectrophotometric determination of vitamin A, which will be discussed.

The preparation of colorimetric standards. FERRIN B. MORELAND. *Biochemical Laby., State Univ. of Iowa, Iowa City.* In colorimetric determinations it is often found when the color is developed that the standard and unknown differ too much for accurate readings. Usually this is remedied by repeating the determination with a different amount of standard or unknown sample. It would be quicker to increase the color by the addition of more standard directly to the paler of the two colored solutions, if it were known that the increase in color intensity were proportional to the amount of added standard. The present investigation has demonstrated the proportionality between increased color and additional standard added after color has been developed according to the usual procedure, as follows: for the determination of phosphate (Fiske and Subbarow), with an error of less than 2 per cent; of creatinine (Folin), with an error of less than 1 per cent; and of ammonia (preformed or from the digestion of nitrogenous material) by nesslerization (Koch and McMeekin) within 5 per cent. Uric acid added after the other reagents in the Christman and Ravwich modification of the method of Benedict and Franke, produced no color; hence the colored solution could not be fortified in this determination.

The heat denaturation of crystalline horse serum albumin. HANS NEURATH, GERALD R. COOPER, JOHN O. ERICKSON and D. GORDON SHARP. *Dept. of Biochemistry and the Dept. of Surgery, Duke Univ. School of Medicine, Durham, N. C.* Salt-free solutions of crystalline horse serum albumin were heated for 30 minutes to 70°C. at pH 7.6 and pH 3.6 and the changes in physical properties were followed by diffusion, viscosity, and electrophoretic measurements on the cooled solutions after the addition of salts to the desired ionic strength (μ). The influence of time of heating was determined by measuring the increase in relative viscosity.

After heating at pH 7.6, the solution consisted of two components when measured at $\mu > 0.09$. Both components differed from the native protein in diffusion constant and electrophoretic mobility. The molecular weight of the major component (90 per cent) was about four times that of the native protein; that of the remainder was considerably higher. At $\mu = 0.02$, the heat-treated protein migrated with a single boundary.

When heated at pH 3.6, the solutions appeared to be monodisperse with respect to both diffusion and electrophoresis at $\mu = 0.1$. While the electrophoretic mobility was the same, the molecular weight was about twice that of the native protein.

The increase in relative viscosity was higher when the protein was heat treated at pH 7.6 than at pH 3.6 but considerably lower than that produced by urea denaturation. It was irreversible at the higher pH and partially reversible at the lower pH.

Urinary excretion of heroin (diacetylmorphine). FRED W. OBERST. *U. S. Public Health Service Hospital, Lexington, Ky.* The chemical structure of heroin suggests a variety of possible urinary excretion products: unchanged heroin, either the 2 or the 6-monoacetyl derivative, or morphine. Certain of these products may be excreted in a conjugated form. The present study was designed to investigate the excretion of heroin in addicted patients and to attempt to determine the chemical nature of the excreted product. Free and bound morphine were determined in urine of 6 morphine addicts before and after complete substitution of heroin for morphine. It was found that both free and bound morphine are present in urine during heroin administration. The amount of free morphine was only slightly decreased, despite the greatly decreased dosage, the replacement dose of heroin being approximately one-third that of

morphine. The percentage excretion was usually considerably higher. No acetylated morphine derivatives were found in this fraction. The amount of bound morphine in urine after heroin administration was considerably less than during the administration of morphine, while the percentage excretion, although variable, approximated that found during morphine administration. No evidence was found that either of the acetyl groups of heroin remains intact during its passage through the body.

Proteinase of wheat gluten. H. S. Olcott and L.A. Sapirstein. *Protein Division, Western Regional Research Laby., Albany, Calif.* An enzymatic degradation of washed wheat gluten in dilute (0.01 to 0.1 N) acetic acid dispersions is demonstrable by an increase in the formol titration. The rapid decrease in viscosity of such solutions previously noted by Cook and Rose (Can. J. Res. **12**: 63, 1935), may also be attributed at least in part to the same enzyme. Both phenomena can be prevented by heating the solutions at 100° for 5 to 10 minutes. No precipitate appears and the salting-out characteristics of the proteins are approximately the same before and after heating, suggesting that the larger portion of the proteins is not denatured by such treatment.

Dispersions of gluten in 10 per cent sodium salicylate solution do not change in viscosity on standing. However, gluten in salicylate is readily digested by dialyzed crude trypsin and papain preparations, hence the wheat flour proteinase differs from these in its lack of activity in salicylate. That the gluten enzyme is inactivated but not destroyed is indicated by its demonstrable presence in acetic acid dispersions of gluten dialyzed free of salicylate.

The viscosity of salicylate dispersions or of heat-inactivated acetic acid dispersions is markedly decreased by reducing agents such as cysteine, thioglycol, and sodium bisulfite, but these substances do not increase the formol titration. These observations are in accord with those of Balls and Hale (Cer. Chem. **13**: 656, 1936) on the effect of reducing substances on wheat gluten.

Further studies on the disposition of injected glucose in a diabetic strain of rats. J. M. Orten, G. Sayers, M. Sayers and A. U. Orten. *Dept. of Physiological Chemistry, Wayne Univ. College of Medicine, Detroit, Mich.* The disposition of intraperitoneally administered glucose was followed in fasted non-diabetic rats (Wistar and "Yale" strains) and "diabetic" rats (Yale strain). The amounts of glucose absorbed, excreted in the urine, stored as liver and carcass glycogen, and present in the liver and entire carcass as "free fermentable" and "total acid hydrolyzable" carbohydrate were determined.

Little difference was found in the peritoneal absorption and renal excretion of glucose in diabetic and non-diabetic rats. However, the storage of glycogen in the liver and carcass was significantly less and the "free" sugar content of the liver and tissues was significantly greater in the diabetic animals. The quantity of total carbohydrate in the liver and carcass of the diabetic rats was less, due undoubtedly to the smaller amount of glycogen. As estimated by calculation, the amount of the injected glucose oxidized (etc.) by diabetic rats was greater than that oxidized by non-diabetic rats.

These results suggest that the "diabetic" tendency of the Yale strain of rats is related to either an impaired ability to store glycogen or to an excessive rate of glycogenolysis. Because of the similarity of certain of these findings to those in epinephrine-injected rats, preliminary experiments have been conducted to study the possible involvement of the adrenal medulla in the diabetic condition. These studies will also be reported.

Excretion of androgens by patients with Addison's disease. Arnold E. Osterberg, Della G. Drips, Edwin J. Kepler and Francis J. Robinson. *Sections of Clinical Biochemistry, Obstetrics and Gynecology, and Medicine, Mayo Clinic, Rochester, Minn.* The urinary excretion of 17-ketosteroids was determined by the Oesting method in 24 patients having Addison's disease. Pooled specimens were employed (average, 72-hr. pool). In a majority of instances, biologic assay of the androgenic potency of extracts of these specimens was carried out with a method developed in this laboratory. In this method, employing immature male rats, the androsterone equivalent of assay material is obtained by reference to three (weighted) criteria: 1, increase in weight of the seminal vesicles; 2, increase in weight of the ventral prostate, and 3, histologic changes in the epithelium of the seminal vesicles. By refering to standard curves for each of these responses, which were obtained by plotting the values of responses obtained at different levels of administration of androsterone, the androsterone equivalent of the material for assay was obtained.

In confirmation of the work of others, we noted reduced levels of excretion of 17-ketosteroids and of androgenic substances in subjects with Addison's disease. The ratio of colorimetric to biologic androgen values appeared to be lower than in normal control subjects or in subjects suffering from a variety of disorders. Administration of desoxycorticosterone acetate was usually followed by increases in the colorimetric values. This was even noted in a female patient who was well beyond the menopause. Biologic assays revealed

that women with severe Addison's disease may excrete androgenic substances.

Utilization by man of the vitamin B complex in fresh yeast. HELEN T. PARSONS, JEAN COLLARD, JOSEPHINE GARDNER, F. M. STRONG and W. H. PETERSON. *Biochemistry Dept. and the Home Economics Nutrition Laby., Univ. of Wisconsin, Madison.* The thiamin, riboflavin and biotin of fresh yeast were found to be very poorly utilized by human subjects, as judged by the lack of any consistent increase in the output of these in the urine after consumption of three yeast cakes per person per day in comparison with the output on the basal diet alone. Thiamin was assayed by the Wong and Harris method and riboflavin and biotin by the microbiological methods of Strong and Snell and of Snell, Eakin and Williams, respectively.

On the other hand heating the yeast to boiling led to a distinct increase in the output of urinary thiamin and riboflavin. Moreover, the fecal content of thiamin was less during the cooked yeast period than during the raw, indicating that the raw yeast which tended to hold onto its thiamin and riboflavin released these for absorbtion to a greater extent after heating. By plating methods, feces were found to contain viable yeast cells after 3 days of raw yeast feeding.

In contrast with these results, biotin appeared to be more firmly bound in the yeast, since urinary assay failed to reveal any striking increase in the excretion of biotin after heated-yeast feeding compared with raw-yeast feeding. Addition of an equivalent amount of biotin to the diet in the form of liver resulted in a very marked increase in the biotin content of the urine. Preliminary results indicate that pantothenic acid in yeast behaves more like biotin than like thiamin and riboflavin when the yeast of the diet is heated to boiling.

A polysaccharide produced by the crown-gall organism. W. H. PETERSON, FLOYD C. MCINTIRE and A. J. RIKER. *Depts. of Biochemistry and Plant Pathology, Univ. of Wisconsin, Madison.* On a sucrose-salts medium, the crown-gall organism *Phytomonas tumefaciens* (Smith and Town) produced a polysaccharide which amounts to 15 to 20 per cent of the sugar consumed. This polysaccharide has been purified until it appears to be a homogeneous substance as judged by diffusion data on the purified material and by constant rotation of fractions of this preparation.

The specific rotation of the polysaccharide is −9 to −10 and of the acetyl derivative +56 to +58.5. Hydrolysis of the polysaccharide gave only glucose. The rate of hydrolysis and shape of the hydrolysis curve suggested a pyranoside structure. The molecular weight of the polysaccharide, calculated from sedimentation and diffusion constants, was 3600 ± 200, which corresponds to about 22 anhydroglucose units per molecule.

Photochemical spectrum of the respiratory ferment in a tissue of the chick embryo. FREDERICK S. PHILIPS (National Research Council Fellow in the Natural Sciences, 1941–42). *Dept. of Physiological Chemistry, Yale Univ. School of Medicine, New Haven, Conn.* The analysis of the spectrum of the respiratory ferment in higher animal tissues by Warburg's photochemical method is rendered difficult by the resistance of the respiration of many tissues to CO-inhibition (Laser). Recent work in this Laboratory on the Pasteur enzyme in retina (K. G. Stern and J. L. Melnick. J. Biol. Chem. **139:** 301, 1941) and on colloidal cytochrome oxidase preparations from heart muscle (J. L. Melnick Science, **94:** 118, 1941) made it desirable to secure information on the spectrum of the respiratory ferment in *intact* cells of higher animals. The *area vitellina* of the chick embryo was chosen since its respiration is susceptible to light-reversible CO-inhibition (F. S. Philips, Anat. Record **78:** Suppl. 77, 1940).

The effect of intense monochromatic radiation on the rate of the oxygen uptake of this tissue from 3-day embryos was studied in 95 per cent CO and 5 per cent O_2 at 24.7°. The determination of the efficiency of 18 wave-lengths between 405 and 597 mμ yielded a relative photochemical absorption spectrum with a steep maximum (Soret band) between 435 and 445 mμ and two lower maxima near 553 and 589 mμ.

While differing in detail from the spectra of the respiratory ferment in microorganisms, of cytochrome-oxidase preparations, and of the Pasteur enzymes in retina and yeast, the spectrum of the respiratory enzyme in chick embryo tissue was likewise that of a *pheohemin protein.* [*This work was aided by a grant to Dr. Kurt G. Stern from the Jane Coffin Childs Memorial Fund for Medical Research.*]

Variations in the concentration of blood constituents following ligation of the hepatic ducts in dogs. LOUIS J. POLSKIN, BERNARD E. SIMON, SAMUEL SKLERSKY, ALBERT E. SOBEL and ALEX WEINERMAN. With the technical assistance of Henry Leonard, Raymond Liebling and George Kraus. *Pediatric Research Laby., Dept. of Biochemistry, and Dept. of Experimental Surgery of The Jewish Hospital of Brooklyn, N. Y.* Although studies have been made of specific changes in the chemical composition of the blood following ligation of the hepatic duct, a correlation of the levels of the various blood constituents has been given little attention. Following ligation of the common hepatic duct on five dogs, significant increases occurred in the per cent *free* and total cholesterol, icterus index, Van den Bergh reaction, esterase,

and phosphatase as early as 18 hours after operation. These changes were more marked at the end of 72 hours. The total lipids rose a few days thereafter. The total lipids and total cholesterol returned almost to the initial values towards the end of the experiment, but the per cent *free* cholesterol remained elevated. The other values in this group remained high. Fecal urobilinogen was absent, or present in traces, within a week after the operation. A gradual decrease in the concentrations of amylase, total protein and fibrinogen was observed. No significant variations were noted in the blood sugar, urea, and inorganic phosphorus. The phospholipid and lipase values were most irregular. The prothrombin clotting time did not rise until the terminal stages.

Two dogs in which two hepatic ducts were blocked showed an increase in the concentration of per cent *free* and total cholesterol, total lipid, Van den Bergh reaction, esterase, and phosphatase. The icterus index, however, remained unchanged. Unlike the similar rises noted in the dogs with complete hepatic blockage, these changes returned to the normal range.

Colorimetric determination of magnesium. MARSCHELLE H. POWER. *Division of Biochemistry, Mayo Foundation, Rochester, Minn.* Recently described methods have been modified so as to simplify the procedure and increase the consistency and accuracy of results. Five cubic centimeters of protein- and calcium-free solution containing 0.01 to 0.10 mgm. of magnesium are transferred to a 15 cc. conical centrifuge tube and treated with ammonium chloride, 8-hydroxyquinoline, and ammonia, essentially according to Hoagland (J. Biol. Chem. **136**: 553, 1940). After one hour or longer at room temperature the precipitate of magnesium hydroxyquinolate is centrifuged and washed, adaptations of the procedures of Hoffman (J. Biol. Chem. **118**: 37, 1937) being used. The supernatant fluids may be removed by decantation without loss of precipitate. The precipitate is dissolved in 2 cc. of 0.05 N HCl, after which 3 cc. of 0.3 M glycine and 1 cc. of 0.5 per cent solution of ferric chloride in 0.01 N HCl are added. The dark green-blue color which forms immediately may be measured by comparison in a colorimeter with appropriate standards, or more advantageously by means of a photoelectric colorimeter with a light filter transmitting maximally at wave lengths 620 to 640 millimicrons.

In an instrument of the latter type, the Klett-Summerson with logarithmic scale, the colored complex corresponding to amounts of magnesium up to 0.05 mgm. may be read directly; deeper colors representing up to 0.10 mgm. of magnesium must be diluted before reading with an appropriate mixture of the reagents used for the initial development of color. The colors obtained are stable, reproducible, and proportional to the amount of magnesium present.

Renal excretion of electrolytes by subjects with Addison's disease. MARSCHELLE H. POWER, FRANCIS J. ROBINSON and EDWIN J. KEPLER. *Division of Biochemistry, Mayo Foundation, and Division of Medicine, Mayo Clinic, Rochester, Minn.* Plasma clearances of urea and electrolytes were determined in 24 subjects with Addison's disease and in 21 control subjects. The periods of study included two of fluid deprivation followed by hourly periods of increasing and decreasing diuresis after administration of water (20 cc. per kg.), while the subjects remained at bed rest.

The type of response of the subjects with Addison's disease differed from that of the control group in many respects, among which may be mentioned: 1, the relatively small diuresis after ingestion of water; 2, diminished clearances of urea, potassium and inorganic phosphorus relative to those of chloride and sodium after ingestion of water; 3, increased ratio of sodium to chloride clearances after ingestion of water; 4, diminished excretion of ammonia associated with consistently acid urines; and 5, less diurnal variation in excretion of electrolytes, especially potassium.

In the control group the clearances of potassium during periods of increasing diuresis tended to be greater than during periods of decreasing diuresis. The administration of desoxycorticosterone acetate to the subjects with Addison's disease was followed by change of the clearances toward normal. However, the magnitude of the diuretic response to the ingestion of water was not proportionately affected.

Comparison of the action of Russell viper venom with that of thromboplastin. ARMAND J. QUICK. *Dept. of Pharmacology, Marquette Univ. School of Medicine, Milwaukee.* Both Russell viper venom and thromboplastin, prepared from brain dehydrated with acetone, convert prothrombin to thrombin in the presence of ionic calcium. Thromboplastin exhibits group specificity, i.e., extracts of avian brain show only feeble activity in mammalian plasma, and vice versa the thromboplastin of mammals acts sluggishly on bird prothrombin. Russell viper venom is equally potent in activating the prothrombin of avian and of mammalian plasma. The activity of venom is markedly increased by the addition of lecithin, whereas that of brain extract is not. Short heating to the boiling point of water diminishes but does not completely destroy the activity of thromboplastin and of venom. The latter is somewhat more labile to heat but a slight stabilizing effect is afforded by the addition of lecithin.

Dehydrated brain extracted with ether loses most of its thromboplastic activity, and neither

the readdition of the ether extract nor the addition of lecithin restores its original potency. The ether extract strongly potentiates Russell viper venom. A mixture of venom and brain extract has a greater activity than either one alone. The further addition of lecithin does not increase the potency of the mixture. Whether the two factors act synergistically or additively is difficult to decide, but probably the action is a combination of both.

Sulfapyrazine. A new chemotherapeutic agent. GEORGE W. RAIZISS, M. SEVERAC and J. C. MOETSCH. *Graduate School of Medicine, Univ. of Pennsylvania, and the Abbott Labs., Philadelphia Division.* Sulfapyrazine (a new derivative of sulfanilamide) is chemically 2-sulfanilaminopyrazine. Sulfapyrazine administered in the diet of mice is rapidly absorbed, giving rise to comparatively high blood concentrations. Toxicity of the drug was studied on mice and rabbits. A good therapeutic effect was observed when the chemical compound was added to the diet of mice infected with pneumococci Types II and III, streptococcus hemolyticus and staphylococcus aureus.

Comparative studies of sulfapyridine, sulfathiazole, and sulfadiazine are also presented.

A bioassay for the effect of the hormones of the adrenal cortex on carbohydrate metabolism. ROGER M. REINECKE. *Division of Biochemistry, Mayo Foundation, Rochester, Minn.* Extracts and crystalline hormones of the adrenal cortex have been standardized by their effect on renal function, on the maintenance of constituents of the blood within normal limits, and on growth. Since no satisfactory bioassay of the effect on carbohydrate metabolism is available, attempts have been made to devise such a method, based on the deposition of glycogen in the liver of fasting, adrenalectomized rats.

Male rats weighing 140 to 160 grams are adrenalectomized on the morning of the first day, and are then maintained for the first and second days either by injections of adrenal cortical extract or by salt. The regular diet is given until the morning of the second day at which time all food is removed. Starting on the morning of the third day the rats are given, at intervals of an hour, seven equal subcutaneous injections of the extract to be assayed. The animals are anesthetized with sodium amytal seven hours after the first injection. The livers are then analyzed for glycogen. If the dose is adjusted so that the glycogen in the liver falls under 1 per cent, the concentrations of glycogen in the liver will be a positive function of the dosage. Under the conditions used untreated ras invariably have less than 0.1 per cent of glycogen in the liver. Maximal deposition of glycogen is less than 2 per cent. The method permits the assay of a solution with unknown activity in terms of a standard.

The clinical determination of urinary androgens. W. A. ROBBIE, A. H. SCHEIN and R. B. GIBSON. *Pathological Chemistry Laby., University Hospitals, The State Univ. of Iowa, Iowa City.* In the course of a study of androgen excretion by patients with prostatic cancer, a simplified method of determining androgenic substances in urine was devised. This method involves less complicated apparatus than that currently employed. The extraction apparatus consists of a reflux condenser and Erlenmeyer flask with ground glass joints. Large or small volumes of urine may be extracted according to the size of the sample. We have found that 250 to 1000 cc. volumes are convenient. After a preliminary hydrolysis with 10 per cent HCl for 15 minutes, one-fifth the urine volume of carbon tetrachloride is added, and the superimposed fluids are refluxed in the flask for two hours on a steam bath. A reextraction after this time shows that no appreciable quantity of androgenic material remains in the urine. A clean separation of the layers is obtained by centrifuging, without the usual bothersome emulsion difficulties. A modified Talbot procedure with Girards Reagent T, to separate the 17-keto steroids from interfering substances, yields samples which, on the addition of 5M KOH and 2 per cent m-dinitrobenzene in alcohol develop colors comparable with solutions of crystalline androsterone treated in the same manner. Either a photo-electric photometer or a visual colorimeter may be employed for this comparison. Dilution with 75 per cent alcohol instead of 95 per cent will prevent the turbidity sometimes noted with higher concentrations of alcohol due to carbonate precipitation. Figures consistent with normal values have been obtained from male laboratory workers.

A comparison of the reaction of carbon suboxide on proteins with that of ketene and the digestion of malonyl albumins by chymotrypsin. WILLIAM F. ROSS and ANN H. TRACY *Chemical Laby. of Radcliffe College, Cambridge, Mass.* Carbon suboxide differs from ketene both in its rate of reaction and in its relative affinity for the amino and phenolic hydroxyl groups of proteins. It reacts at an approximately equal rate with these two radicals, all present in serum albumin being covered after the addition of three equivalents. With ketene, on the other hand, the addition of six equivalents is required before the amino groups have completely reacted, at which time 40 per cent of the phenolic groups are still free.

The malonyl tyrosine ester linkage is unstable in aqueous solutions of nearly neutral pH even at low temperatures. Of the malonic acid bound to tyrosine in malonyl serum albumin, 50 per cent is

liberated after three weeks at a pH of 5.1 and at 2°, and the hydrolysis takes place at a much more rapid rate at 37.5°.

Study of the chymotryptic digestion of malonyl egg and serum albumins indicates that at least two factors are involved. Initial digestion of these treated proteins may or may not be greatly accelerated above that of the corresponding native proteins. The total number of peptide linkages split, however, is less with the malonyl proteins. Change in the physical condition of the molecule influencing its availability to the enzyme, and the blocking of groups specific for chymotryptic splitting may be responsible for these differences.

Changes in mineral composition of rat lenses with galactose cataract. PETER W. SALIT, KENNETH C. SWAN and WILLIAM D. PAUL. *Depts. of Ophthalmology and Internal Medicine, College of Medicine, The State Univ. of Iowa, Iowa City.* In order to throw additional light on the nature of the human cataract, rat lenses which were the seat of galactose cataracts were analyzed for their water content, ash, and the most important cations and anions that the animal tissue is known to contain. Similar analyses were carried out on normal rat lenses for the purpose of comparison. The rats with galactose cataracts were furnished by Dr. Helen Mitchell, of Amherst College.

The water content of the lenses increased from 59.71 per cent in normal lenses to 73.81 and 80.59 per cent in incipient and complete cataracts, respectively. The total ash rose from 0.617 per cent in normal lenses to 0.862 and 0.923 per cent in cataractous lenses, as estimated by wet weight, and from 1.034 to 1.168 and 1.144 per cent as estimated by water content, respectively. Potassium, phosphate (PO_4), and chlorine decreased in cataractous lenses, but sodium, calcium, sulfate (SO_2), and carbonate (CO_3) increased.

The total content of cation was more uniform in distribution among the three groups of lenses than that of anions, both with respect to wet weight and water content. The degenerative processes of lens tissue seem to be, therefore, more intimately connected with changes in the anions than in the cations.

In general, the changes were similar to those which occur in human senile cataracts.

Porphyrinuria as a sign of lead poisoning. Observations on dogs. KURT SALOMON and GEORGE R. COWGILL. *Depts. of Physiological Chemistry and Pharmacology, Yale Univ. School of Medicine, New Haven, Conn.* In humans and rabbits porphyrinuria is one of the objective signs of lead poisoning. Other investigators who have failed to observe porphyrinuria in lead poisoned dogs have suggested that the metabolism of respiratory pigments in lead poisoned carnivores is different in some important respect from that in other species.

We have followed, over about five months, urinary porphyrin excretion (protoporphyrin and coproporphyrin), appearance of stippled erythrocytes, and concentrations of blood hemoglobin and lead in two puppies (a control, and one receiving lead) and two adult dogs. The animals were fed an artificial diet low in both calcium and iron-porphyrin compounds. Urinary porphyrins were extracted by the Dobriner modification of Fischer's acetic acid ether method from urine collected over ten-day periods; final measurement was made with an Evelyn photoelectric colorimeter; blood examinations and analyses were made on the tenth day of the period. In all cases porphyrinuria occurred only after prolonged feeding of large doses of lead. In the adult animals the urinary porphyrin excretion was thus increased tenfold. The chemical configuration of the excreted porphyrin was investigated. No time relationship between the onset of porphyrinuria and anemia was noted. Lead blood level was elevated at an early stage of the lead exposure. Stippling (three stippled cells per thousand) occurred in only one dog. The observed porphyrinuria was a late rather than early sign of lead intoxication. Evidently the canine species is no exception as regards the effect of lead in producing porphyrinuria.

The effect of previous diet on the basal metabolic rate of hypophysectomized and normal male rats. LEO T. SAMUELS, ROGER M. REINECKE and KENNETH L. BAUMAN. *Division of Physiological Chemistry, Dept. of Physiology, Univ. of Minnesota, Minneapolis.* When the body weights of hypophysectomized male rats were maintained by stomach tube feeding, the basal metabolic rates determined by the open-circuit Haldane technique were similar to those of untreated rats maintained in the same way. When the animals were allowed to eat the same diets *ad libitum*, however, the hypophysectomized rats on a high fat regime had basal metabolic rates 23 per cent less than similarly fed controls, while hypophysectomized rats fed a high-carbohydrate mixture had basal rates 30 to 35 per cent less than the controls. These results have been correlated with differences in liver composition. The thyroids were also investigated histologically. The maintenance of fat reserves in the liver would appear to be an important factor in maintaining the metabolic rate of hypophysectomized rats. [*This investigation was supported by a grant from the Committee on Research in Endocrinology, National Research Council.*]

The effect of variations in protein intake on the urinary excretion of riboflavin, nicotinic acid, pyridoxin and thiamin in dogs. HERBERT P. SARETT, J. RAYMOND KLEIN and WILLIAM A.

PERLZWEIG. *Dept. of Biochemistry, Duke Univ. School of Medicine, Durham, N. C.* Three growing dogs were fed various mixtures of vitamin free casein (Labco), beef suet, sucrose, and starch, supplemented with 0.7 gram salt mixture, 0.5 gram cod liver oil, and 1 gram of dried brewer's yeast per 100 calories. The urinary excretion of riboflavin during periods of low protein intake: e.g., 1 gram per kgm., was about five times that found during periods of moderate and high protein intake; e.g., 7 and 12 gram per kgm. The urinary excretion of total nicotinic acid (acid hydrolyzable derivatives and trigonelline) was found to vary with the protein intake in a similar fashion but to a lesser extent. The fractions of subcutaneously injected doses of riboflavin (100 1 per kgm.) excreted in the urine during periods of low, medium and high protein intake were 70 per cent, 27 per cent, and 3 per cent, respectively. The fractions of doses of pyridoxin and nicotinamide accounted for in this way were highest on the low protein diet, but did not differ on medium or high protein diets. The urinary excretion of thiamin and the response to test doses could not be correlated with the variations in protein intake. These findings were independent of variations in carbohydrate, fat, and total caloric intake and were reproducible for all three levels of protein intake in each dog.

Pantothenic acid deficiency in dogs. A. E. SCHAEFER, J. M. MCKIBBIN and C. A. ELVEHJEM. *Dept. of Biochemistry, Univ. of Wisconsin, Madison.* Weanling mongrel puppies were placed on a ration consisting of sucrose 66, casein 19, cod liver oil 3, cottonseed oil 8, and salt mixture 4 per cent. This was supplemented with synthetic thiamine, riboflavin, nicotinic acid, pyridoxine and choline. Rapid growth resulted for 3 to 5 weeks whereupon a sudden acute deficiency resulted, usually characterized by prostration or coma, rapid breathing, accelerated heart rate, and vomiting. The blood sugar in these dogs was usually lowered, blood chlorides slightly depressed, and the non-protein nitrogen was increased. Serum calcium and inorganic phosphorus were normal. The injection of calcium pantothenate and glucose produced rapid alleviation of the acute symptoms, but growth was frequently not resumed until 1 to 2 weeks after the injection. Blood sugar, chlorides, and non-protein nitrogen returned to the normal levels of the control dogs receiving pantothenic acid.

Necropsies of dogs dead of the deficiency revealed mottled fatty livers, hemorrhagic kidneys and thymus glands, and often intussusception in the lower ileum or involving the duodenal cap and pyloric stomach. Adrenals usually appeared normal. Much of the same picture was seen in older dogs receiving this ration. Greying of the hair was seen occasionally on this ration but this has been observed in dogs receiving adequate pantothenic acid. The requirement for the vitamin is tentatively placed at about 100 gammas of the calcium salt per kilogram of body weight per day.

Bacterial oxidation of cholic acid. L. H. SCHMIDT, H. B. HUGHES and WILLARD M. HOEHN. *Inst. for Medical Research, Christ Hospital, Cincinnati, O., and George A. Breon and Co., Kansas City, Mo.* The rapid conversion of cholic acid into derivatives that give negative Gregory—Pascoe and Pettenkofer reactions is brought about by growing cultures of an organism isolated from the cecum of the guinea pig and having the cultural characteristics of *Alcaligenes fecalis*. This conversion occurs only under aerobic conditions, suggesting that an oxidation of cholic acid might be the process involved.

Qualitative and quantitative studies of the above reaction have shown that oxidation of cholic acid does occur with the formation of various keto-cholanic acids. The end product of the reaction is 3,7,12-triketo-cholanic (dehydrocholic) acid, which has been isolated in pure form (M.P. 237–9°) in a yield of 85 per cent of that obtainable theoretically from the quantity of cholic acid in the reaction mixture. At least two other keto-cholanic acids are formed as intermediary oxidation products. They are 7-keto-3,12-dihydroxy-cholanic acid and 7,12-diketo-3-hydroxy-cholanic (reductodehydrocholic) acid. These mono- and diketo-acids have not yet been isolated in pure form. Their identity has been established, however, by (1) the formation of the mono- and di-semicarbazones of the methyl esters of the crude keto-acids isolated from the bacterial digest; (2) the separation of the mono- and di-semicarbazones on the basis of solubility in methanol; and (3) the Wolff-Kishner reduction of the semicarbazones to hydroxycholanic acids—the mono-semicarbazone yielding 3,12-dihydroxycholanic (desoxycholic) acid, and the di-semicarbazone yielding 3-hydroxy-cholanic (lithocholic) acid.

Carbonic anhydrase. D. A. SCOTT and A. M. FISHER. *Connaught Labs., Univ. of Toronto, Canada.* A method is described for the production of carbonic anhydrase preparations having an activity of about 10,000 units per mg. The product is prepared from material described in an earlier publication (D. A. Scott and J. R. Mendine, J. Biol. Chem. **140:** 445, 1941) by treatment with potassium phosphate, chloroform and dialysis. The preparation contains 0.2 per cent of zinc and 15.8 per cent of nitrogen. Thin plate-like crystals separate from alcohol-ether solutions upon treatment with ammonia. The crystals contain at least 80 per cent of the activity of the solutions from which they are prepared. Examination under a polarizing microscope shows that the plates are isotropic.

Influence of alpha-tocopherol and unsaturated fatty acids upon the utilization of vitamin A. W. C. SHERMAN. *Laby. of Animal Nutrition, Alabama Polytechnic Inst., Auburn.* The antagonism between linoleic and linolenic acid esters and carotene was prevented by feeding the unsaponifiable fraction of soybean oil to vitamin A deficient rats, but not by feeding choline, ethanolamine, lecithin, cephalin, hydroquinone, or catechol. The feeding of α-tocopherol protected against this antagonism.

In vitro tests showed that carotene was rapidly destroyed by methyl linolate in the absence of suitable antioxidants. There was, however, no direct destruction of carotene by the methyl linolate in the feeding tests, since the yeast and casein of the basal diet were found to possess stabilizing properties. Hydroquinone and catechol also stabilized carotene with methyl linolate *in vitro*, although they were entirely inactive when fed.

The destruction of carotene by unsaturated fatty acids apparently occurs mainly in the intestines, since feeding the carotene and methyl linolate on alternate days removed the antagonism. Furthermore, methyl linolate fed during the depletion period did not affect the time required to produce vitamin A deficiency.

To be protective, α-tocopherol had to be fed with the carotene and methyl linolate. Protection was not obtained by feeding the alpha-tocopherol during the depletion period, or by continued feeding of alpha-tocopherol on one day and carotene with methyl linolate on the following day.

Direct proof of intestinal destruction of carotene by methyl linolate and protection by alpha-tocopherol was obtained from analyses of the gastro-intestinal contents of rats after they had received various combinations of carotene, methyl linolate, and alpha-tocopherol.

Intestinal synthesis of biotin and growth of rats on a low-biotin diet. GILBERT M. SHULL and EDWARD NIELSEN. *Dept. of Biochemistry, Univ. of Wisconsin, Madison.* In the course of developing a microbiological assay for biotin utilizing *Lactobacillus casei* ϵ, it was found that 0.3 per cent hydrogen peroxide inactivated biotin for the rat as well as for this organism.

This peroxide inactivation of biotin was utilized in preparing a biotin-low synthetic ration for rats of the following composition: sucrose 73, casein 18, corn oil 5, salts 4 per cent. Rats maintained on this ration with a supplement containing all of the known vitamins except biotin failed to show any deficiency symptoms and exhibited steady gains in weight, although the ration contained only 0.3 mγ biotin per gram. Assays of the feces of the rats on this ration indicate a marked intestinal synthesis.

The effect of sulfaguanidine on the intestinal synthesis of biotin will be discussed.

Effect of hemins on the catalyzed oxidation of brain brei and phospholipids. FRIEDA PANIMON SIMON, M. K. HORWITT and R. W. GERARD. *Biochemical Research Laby., Elgin State Hospital, Elgin, Ill., and the Dept. of Physiology, Univ. of Chicago, Ill.* Following our observation (F. Panimon, M. K. Horwitt and R. W. Gerard. J. Cell. and Comp. Physiol. **17: 17, 1941**) that hemoglobin depresses orthophenanthroline catalysis of brain brei (mainly phospholipid) oxidation, we further investigated this phenomenon using less complex systems. Oxidation of more purified phospholipids and of oleic and linoleic acids, as well as of brain brei, was followed (Warburg technique) in the presence of ferrous orthophenanthroline as the principal catalyst. Hemoglobin, as low as 8×10^{-6} M, catalyzes oxidation of linoleic acid tenfold and of phospholipid twofold, but at the same time approximately halves the extra oxygen uptake induced by iron orthophenanthroline. Cyanide only slightly or temporarily inhibits the iron-dye effect on oxidation of the unsaturated fatty acids, does not affect the hemoglobin catalysis of this oxidation, and partially counteracts hemoglobin inhibition of the iron-dye.

Of the other hemin compounds tested, cytochrome c (4×10^{-7} M) also catalyzes oxidation of linoleic acid (fivefold), but does not materially affect iron orthophenanthroline catalysis. Catalase (3×10^{-10} M to 3×10^{-7} M) has little or no effect on the autoxidation of fatty acid or phospholipid or on their hemoglobin-catalyzed oxygen uptake. Hexylresorcinol prolongs the induction period of linoleic oxidation in the presence of iron orthophenanthroline.

An enzymatic sulfur exchange between cysteine and radio-active sulfides. C. V. SMYTHE and D. HALLIDAY. *Depts. of Chemistry and Physics, Univ. of Pittsburgh, Pa.* An enzyme system that forms hydrogen sulfide, ammonia, and pyruvic acid from cysteine was previously described. In order to test whether or not the formation of the hydrogen sulfide is reversible, the reaction has now been carried out in the presence of added hydrogen sulfide containing radio-active sulfur (kindly furnished by the Radiation Laboratory of the University of California). The progress of the reaction was followed by the ammonia formation. When approximately one quarter of the cysteine had formed ammonia, the reaction was stopped, and the remaining cysteine was isolated via the cuprous mercaptide. After removal of the copper, the cysteine was oxidized, and the resulting cystine was isolated, purified, and characterized. Tests for radio-activity were made on the cystine and on the sulfur of the cystine after it had been converted to barium sulfate. The samples were

tested by placing them inside of a Geiger Counter filled with helium at atmospheric pressure. Samples of cystine isolated from different experiments showed counts of 400 to 2300 per minute per milligram of cystine. The theoretically possible count, if there had been complete exchange between the cysteine sulfur and the sulfide and if no other reaction had occurred, was 33,000. The result demonstrates that the formation of hydrogen sulfide from cysteine under the experimental conditions is a reversible process. The reaction furnishes a convenient method of preparing cysteine containing radioactive sulfur.

Substrate competition in the utilization of ketone bodies. MICHAEL SOMOGYI and T. E. WEICHSELBAUM. *Laby. of the Jewish Hospital of St. Louis, Mo.* Edson has shown that in liver slices, carbohydrates are preferentially oxidized when competing with fatty acids for the oxidizing enzyme system of the tissue. Mirsky *et al.* described such substrate competition in the perfused heart.

We observed the same phenomenon in diabetic patients with impaired liver functions. Such patients, when fed glucose during moderate degrees of ketosis, showed a rise of ketonemia (after some initial drop) above the basal level. We reproduced the phenomenon in dogs. By combination of subtotal pancreatectomy and high fat diats, fatty infiltration of the liver was brought about, a condition which diminishes the rate of glycogen formation (glucose phosphorylation) and, in consequence, of glucose utilization in the dog liver. Animals thus prepared developed mild ketosis. Glucose feeding produced in them, like in our diabetic patients, an appreciable increase of the ketonemic level after variable intervals following glucose feeding. Since the ketonemic level represents the difference, ketones produced in the liver minus ketones utilized in the peripheral tissues, we ascribe the rise in the ketonemic level to a diminished rate of utilization in the peripheral tissues, when an ample supply of carbohydrate is available.

It has been recognized in recent years that carbohydrates exert no ketolytic effect in the organism and that the phrase: "fats burn only in the fire of carbohydrates" is unfounded. Now experimental evidence is accumulating to demonstrate the contrary, namely that carbohydrates inhibit the burning of ketone bodies.

A study of the cholesterol of the blood serum in monkeys. WARREN M. SPERRY, JOSEPH W. JAILER and V. A. STOYANOFF. *Dept. of Biochemistry, the New York State Psychiatric Inst. and Hospital and Columbia Univ., New York City.* The concentration of total and free cholesterol in the blood serum was determined at frequent intervals in 10 female monkeys, subjected to various experimental procedures, during periods up to 7 months in length. From these continuous records the following results have been obtained: 1. With one exception, in 17 experiments no significant change in total cholesterol concentration occurred during the 24 hours following the feeding of 2 to 5 eggs or egg yolks. 2. However, when the egg feeding was continued for 3 additional days a rise in total cholesterol became evident, usually after 48 hours, and reached the maximum (the increase in 12 experiments averaged 58 per cent) within 96 hours in most instances. 3. A consistent rise, averaging 40 per cent, in the ratio of combined to free cholesterol accompanied the increase in total cholesterol concentration following egg feeding. Although the increase in total cholesterol was largely in the combined cholesterol fraction, with one exception a definite, but usually small, increase in free cholesterol concentration also occurred. 4. A decrease (average 45 per cent in 4 experiments) in total cholesterol concentration followed administration of 0.25 mg. thyroxine daily for 10 days. Normal, spayed, and thyroidectomized monkeys responded similarly in all the foregoing experiments. 5. Inconsistent changes in total cholesterol concentration followed thyroidectomy in normal and spayed monkeys. [*Aided in part by a grant from the Rockefeller Foundation, administered by Dr. Philip E. Smith.*]

X-ray diffraction studies in derivatives from lipids of tubercle bacilli. II. MONA SPIEGEL-ADOLF and GEORGE C. HENNY. *Dept. of Colloid Chemistry, D. J. McCarthy Foundation and the Dept. of Physics, Temple Univ. School of Medicine, Philadelphia, Pa.* Our x-ray diffraction studies of derivatives from lipids of tubercle bacilli have been recently extended to tuberculostearic acid and phthioic acid. Both substances were kindly provided by Professor R. J. Anderson of Yale University.

Since the melting points of these substances are 10° and 20° respectively, their x-ray diffraction patterns taken at room temperature showed only one amorphous ring of nearly identical diameter. The occurrence of non-specific rings was avoided by keeping the thickness of the layer below the critical value of non-specific rings given by Trillat. In order to get some orientation while avoiding chemical changes, the substances were frozen in the exposure cells. Since polymorphism has been described in aliphatic acids, the temperature was controlled by thermocouples. The x-ray diffraction patterns obtained in this way showed five sharp rings in tuberculostearic acid. The main spacings evaluated according to the Hanawalt method were at 4.76, 3.98, 12.39 Å. In phthioic acid there were three lines corresponding to spacings of 4.10, 4.66, 11.7 Å.

A comparison of these findings with those of analogous studies made on stearic and cerotic

acids respectively was established. Although cerotic acid was not a pure preparation, both x-ray diffraction patterns were very similar (main spacings at 3.98, 4.35 and 3.58 Å). The differences between these patterns and the ones of tuberculostearic and phthioic acids were quite conspicuous. The latter case, especially, seems to add new evidence to our previous findings about the influence which branching of a chain apparently has on the x-ray diffraction patterns.

Catalysis by anions of the hydrolysis of amide and peptide bonds in proteins. JACINTO STEINHARDT. *Research Laby. of the Textile Foundation at the National Bureau of Standards, Washington, D. C.* The rates of hydrolysis by dilute acids of both a dissolved protein (egg albumin) and an insoluble protein (wool) depend not only on the temperature and acidity but also on the acid used. When hydrolyzed at 65° by certain strong monobasic acids of high molecular weight, the amide and peptide bonds of these proteins are broken 60 to 70 times faster than when they are hydrolyzed with hydrochloric acid. Even among the common mineral acids, large differences appear. These differences in hydrolytic effect parallel differences in the affinities of the anions of the acids for protein. Further reasons for identifying this effect with the anions are: *a*, the attainment, with anions of high affinity, of a maximum rate of amide hydrolysis at relatively low concentrations, stoichiometrically equivalent to the sum of the amino plus the amide groups and *b*, failure to observe a similar limiting anion concentration or maximum rate of hydrolysis of the much more numerous peptide groups. In general, amide hydrolysis is catalyzed more strongly than peptide hydrolysis. Thus, by keeping the concentration of the most favorable anion within the stoichiometric limit, amide groups may be rapidly hydrolyzed without extensive general hydrolysis of the protein.

Despite great differences in physical state and resistance to enzymes, both proteins are hydrolyzed at comparable rates. The maximum catalytic effect of anions obtained with egg albumin stands about midway, on a logarithmic scale, between the effects produced by hydrochloric acid alone and by hydrochloric acid plus the same weights of crystalline pepsin.

Further studies on the metabolism of benzyl chloride in the rat. JAKOB A. STEKOL. *Dept. of Chemistry, Fordham Univ., New York, and the Biochemistry Dept., Vanderbilt Univ. Medical School, Nashville, Tenn.* We demonstrated some time ago the conversion of benzyl chloride to N-acetyl-S-benzyl-*l*-cysteine in the rat, rabbit and dog. We have now investigated in some detail the growth inhibition of young rats by benzyl chloride and other related substances on diets of low casein content. We were particularly interested to ascertain whether the inhibition of growth by benzyl chloride was due to the loss of cysteine to the rat as a result of the benzylmercapturic acid formation from benzyl chloride. Several groups of male rats, 30 days of age, were fed 8 per cent casein diet alone or supplemented with the following substances: benzyl chloride, 0.5 per cent; S-benzyl-*l*-cysteine, one per cent; N-acetyl-S-benzyl-*l*-cysteine, one per cent; benzoic acid, one or two per cent; and benzoyl chloride, 0.5 per cent. Rats receiving benzyl chloride ceased growing immediately, in some cases loss in weight was observed. *l*-Cystine or *dl*-methionine, added to the benzyl chloride diet, promptly induced resumption of growth. S-benzyl-*l*-cysteine, benzoic acid, or benzoyl chloride had no effect on the growth of rats. N-Acetyl-S-benzyl-*l*-cysteine checked the growth of rats by about 50 per cent. Cystine or methionine counteracted the inhibition of growth by benzylmercapturic acid.

Loss of cystine as a result of detoxication of a substance (mercapturic acid formation) does not appear to be the sole factor responsible for the observed growth inhibition on a low casein diet.

On alcoholic fermentation as a heavy metal catalysis. KURT G. STERN (with the technical assistance of Jane Maurer). *Dept. of Physiological Chemistry, Yale Univ. School of Medicine, New Haven, Conn.* Fermentation, like respiration and the Pasteur reaction, is a heavy metal catalysis (Warburg, Lipmann, and others). The present experiments were undertaken to obtain further information on the identity of the heavy metal catalyst essential for alcoholic fermentation.

The rate of fermentation of fructose by cell-free maceration extracts from dried Brewers bottom yeast (Hull) was measured manometrically in the presence of reagents forming stable complexes with Fe or Cu. The inhibition of fermentation by o-phenanthroline (cf. Dische, C. R. Soc. Biol. **133**: 380, 1940) could be confirmed. Pyrophosphate and α,α'-dipyridyl, which likewise form complexes with ferrous iron, and 8-hydroxyquinoline, thiourea, and salicylaldoxime, which have a high affinity for copper ions, were ineffective even in high concentrations. Diethyldithiocarbamate and rhodanine, in high concentrations, produced some inhibition.

The yeast maceration extracts contained, on the average, 7.7 micrograms of Fe per cc., more than one half of which was dialyzable. A large fraction of the non-dialyzable Fe was found in the protein precipitate forming upon prolonged dialysis. The extracts contained prophyrin-bound iron as hemochromogen. The two absorption bands, at 580 and 555 mμ, which showed a blue shift of about 5 mμ relative to the corresponding bands in the intact yeast cells, represented the

α-bands of cytochrome a_1 and b_1 components. The quantitative relationship between the protein-linked and the prophyrin-bound iron remains to be established. [*This work was aided by a grant from the Jane Coffin Childs Memorial Fund for Medical Research.*]

The metabolism of ricinoleic acid. W. C. Stewart and R. G. Sinclair. *Dept. of Biochemistry, Queen's Univ., Kingston, Canada.* These experiments were carried out to determine whether ricinoleic acid, *12-hydroxy-$\Delta^{9:10}$-octadecenoic acid*, can be utilized for phospholipid synthesis within the animal body. Rats were fed for several weeks on a diet containing castor oil 48.4, casein 28.1, yeast 14.4, salt mixture 6.3, corn oil 1.4, and cod liver oil 1.4 per cent. During the first few days the rats lost weight; thereafter the body weight either remained constant or increased somewhat. There was no evidence of non-absorption of the castor oil.

Ricinoleic acid was determined by acetylation of the methyl esters of the fatty acids in the phospholipids and neutral fat. The phospholipids of the small intestine, liver, and skeletal muscle contained little, if any, ricinoleic acid. The neutral fat of these same organs did contain ricinoleic acid, although the percentage present was much lower than that in castor oil.

It would appear, therefore, that ricinoleic acid is excluded from participation in the synthesis of phospholipids, even in the intestinal mucosa; and that ricinoleic acid is rapidly metabolized after its absorption, either by combustion or by conversion into non-hydroxylated fatty acids.

The estimation of cystine and cysteine together. M. X. Sullivan, W. C. Hess and H. W. Howard. *Chem-Medical Research Inst., Georgetown Univ., Washington, D. C.* In the study of viruses, hormones, and denatured proteins, it has become of great interest to estimate cystine and cysteine in the presence of each other. Consideration will be given to the application of the Sullivan reaction to various mixtures of cysteine and cystine, and data will be given to show that, with proper attention to the purity of the cystine and cysteine standards, it is relatively easy to estimate quantitatively the cystine or cysteine singly or *in toto* in any mixture.

The manometric measurement of acid production by cells and tissues in retentive media. William H. Summerson. *Dept. of Biochemistry, Cornell Univ. Medical College, New York City.* The direct manometric measurement of acid production (in terms of the decomposition of bicarbonate) by cells and tissues under aerobic conditions in a medium such as blood serum, which in addition to bicarbonate contains other buffers as well, is somewhat difficult since a variable portion of the acid produced is neutralized ("retained") by the non-bicarbonate buffers and does not register on the manometer. Various procedures (Dixon. Biochem. J. **31:** 924, 1937; Dickens. Biochem. J. **33:** 230, 1939) have been proposed for determining the amount of retained acid under these conditions. These procedures have been subjected to a theoretical and experimental evaluation, the results of which will be presented. Particular attention has been directed to the shape of the retention-pH curve for media of high and low buffering power; to the influence of vessel constants and of metabolic CO_2 on the correction for retention; and to the use of the author's constant-volume differential manometer (Summerson. J. Biol. Chem. **131:** 579, 1939) for measurement of aerobic acid production in retentive media.

Thiamin and riboflavin metabolism. Barnett Sure and Zenas Ford, Jr. *Dept. of Agricultural Chemistry, Univ. of Arkansas, Fayetteville.* Thiamin utilization was studied on 8 male albino rats, weighing about 180 gm. each, on daily doses of 20 μg, 50 μg, and 100 μg, the latter dose in the presence and absence of 20 mg. daily of ascorbic acid. Determinations were made with the Pfaltz and Bauer fluorophotometer, using a modification of the Hennessey and Cerecedo procedure (D. J. Hennessey and L. R. Cerecedo, J. Am. Chem. Soc. **61:** 179, 1939). The results show a progressive increase in digestibility and utilization with increased thiamin doses. The per cent utilization for the 20 μg, 50 μg, and 100 μg doses were 75.4, 82.7, and 90.3 respectively. Ascorbic acid produced a 4.8 per cent decrease in thiamin utilization.

Thiamin depletion produces tremendous increases in riboflavin urinary excretion, as determined by fluorophotometric measurements on 5 pairs of animals. No noteworthy changes were observed during the first two weeks of depletion, but during the last two weeks the thiamin deficient animals excreted in the urine four times as much riboflavin as the controls, the former 58.4 per cent, and the latter 14.6 per cent of the total riboflavin intake.

An average daily dose of 0.53 mgm. of synthetic thyroxin produced in 17 days a three-fold urinary excretion of the daily 50 μg riboflavin intake. A 20 mgm. daily supplementation of ascorbic acid to a 100 mgm. daily thiamin dose accentuated the urinary riboflavin excretion in hyperthyroidism. These findings were obtained on 4 pairs of male rats.

The metabolism of acetic acid. Marian E. Swendseid, Allan Hemingway, Richard H. Barnes and A. O. Nier. *Dept. of Physiological Chemistry, The Medical School, Univ. of Minnesota, Minneapolis.* Metabolism studies have been carried out with acetic acid containing an excess complement of the stable isotope of carbon, C^{13}, in the carboxyl carbon atom.

Sodium acetate containing excess C^{13} was given by subcutaneous injection to fasting and fed mice. In both experimental conditions, heavy carbon appeared within the first half hour in the respired carbon dioxide, indicating a rapid oxidation of the acetic acid carboxyl group.

The respired heavy carbon dioxide, observed over an eight hour period, differed in both the rate of excretion and the total amount in the fasting and fed mice. The maximum rate of heavy carbon excretion was attained earlier in the experimental period with the fed mouse. However, more heavy carbon dioxide was expired by the fasting mouse.

As it appeared that in the animal fed acetic acid was stored in some form, sodium acetate containing excess C^{13} was fed to rats which received, simultaneously, a high carbohydrate-low fat diet following a period of fasting. Acetic acid conversion and storage under these conditions of rapid glycogen and fat synthesis will be discussed.

Further studies employing radioactive sulfur. HAROLD TARVER and CARL L. A. SCHMIDT. *Division of Biochemistry, Univ. of California Medical School, Berkeley.* We now prepare methionine* (the asterisk indicates the compound contains radioactive sulfur) by a modified Hill and Robson synthesis, using benzyl mercaptide* in place of methyl mercaptide.

Elementary S* was fed orally to rats either as a single dose or as daily doses over a period of one week. The organic sulfur from the proteins of these animals showed no radioactivity.

Methionine* has been fed to starved bile fistula dogs fed cholic acid. Pure taurine was isolated from the bile and the decomposition points and crystalline forms of both the taurine and β-naphthalenesulfo-taurine were the same as those of the pure substances. The sulfur in the taurine was radioactive.

One day after feeding methionine to a dog, the tissue proteins were examined. Methionine-S obtained after separation of cystine from liver protein showed a slightly greater activity than the cystine-S.

In the proteins, the order of activity per unit of organic sulfur was as follows: intestinal mucosa, liver, plasma proteins, pancreas, kidney, lungs, testes, voluntary and involuntary muscle. Bile protein showed a higher activity than any of the above proteins. [*Aided by grants from the Rockefeller Foundation and Eli Lilly and Company.*]

The effect of bile salts on chromodacryorrhetic action of acetylcholine. SHIRO TASHIRO. *Dept. of Biological Chemistry, Medical College, Univ. of Cincinnati, Cincinnati, O.* Intraperitoneal injection of 4 mgm. of acetylcholine iodide (all dosages were per 100 grams body wt.) always cause chromodacryorrhea (bloody tear production) in noneserinized rats. A similar injection did not produce chromodacryorrhea if the animal had received a previous injection (5 to 10 minutes) of 20 to 30 mgm. of mixed bile salts (Na glycocholate and taurocholate, Fairchild). This failure to produce bloody tear cannot be attributed to the direct effect upon Harder's glands because the bile salts cannot check the chromodacryorrhetic action of carbamylcholine (Doryl, Merck) with non-eserinized rats, nor that of acetylcholine with eserinized rats. If we assume that bile salts would accelerate the action of choline-esterase, these findings would become understandable, but no such assumption could be substantiated in this laboratory so far as the effect of bile salts upon choline-esterase of serum is concerned. The possibility that the bile salts may set the enzyme free from tissues has not been investigated. We have no evidence to indicate that bile salts might have caused non-enzymic hydrolysis of acetylcholine under the experimental conditions employed. The possibility of a bile salt effect on peripheral but not on ganglionic transmission, has not been determined.

The conversion of casein into a methionine and tryptophane deficient protein by an oxidative treatment. GERRIT TOENNIES. *Lankenau Hospital Research Inst., Philadelphia, Pa.* When it appeared that the sulfone of methionine (J. J. Kolb and G. Toennies, J. Biol. Chem. **140:** 131, 1941) displays neither growth-supporting nor toxic properties (M. A. Bennett, J. Biol. Chem. **141:** 573, 1941) the possibility of removing methionine from the dietary essential protein constituents by means of oxygenation of the protein molecule appeared worth examining.

Oxidation of methionine to sulfone resulted from activation of hydrogen peroxide by molybdate. The latter involves formation of a peracid (permolybdic acid). Proteins are soluble in formic acid. Formic acid combines with hydrogen peroxide to form the highly active performic acid. On the basis of such considerations we have dissolved casein in formic acid, added hydrogen peroxide to the solution, and after allowing varying times for reaction, reprecipitated the protein by diluting with water and neutralizing the formic acid with ammonium hydroxide. The isolation was completed by washing with water and methanol and vacuum drying at 70°. Modified casein has thus been obtained, in eighty per cent yield, which is substantially identical with the parent protein in total nitrogen, threonine and serine content. Tryptophane and (according to chemical (cf. T. F. Lavine, these Proceedings) and biological (M. A. Bennett and G. Toennies, these Proceedings) evidence) methionine are substantially absent, while a small fraction of the original cystine appears to remain intact. The name oxy-

casein is suggested for protein derived from casein in which methionine and tryptophane have been selectively destroyed by oxidation.

Purification, kinetics and activity measurement of liver arginase. DONALD D. VAN SLYKE and REGINALD M. ARCHIBALD. *Hospital of The Rockefeller Inst. for Medical Research, New York City.* 1. *Purification:* Hunter's (A. Hunter and J. A. Dauphinee J. Biol. Chem. **85**: 627, 1930) glycerol extract was activated and stabilized by heating at 58°C. for 20 minutes with 1/20th volume of 20 per cent aqueous solution of $MnCl_2 \cdot 4H_2O$. The filtrate from this was fractionated at 0°C. with acetone. Electrophoretic measurements indicated that arginase had the mobility of a component which comprised 35 per cent of the total preparation and an isoelectric point near pH 6.6.

2. *Kinetics:* Arginase follows the general formula of Henri (V. Henri, Ztschr. f. physikal. Chem. **39**: 194, 1902), Michaelis and Menten (Michaelis and M. L. Menten, Biochem. Z. **49**: 333, 1913) and Van Slyke and Cullen (D. D. Van Slyke and G. E. Cullen, J. Biol. Chem. **19**: 141, 1914) for hydrolytic enzymes. With a stabilized enzyme preparation the formula derived by Van Slyke and Cullen for urease applies as long as the concentration of the product ornithine remains insignificant.

3. *Activity measurement:* Using high substrate concentration, the amount of substrate decomposed is directly proportional to the amount of enzyme present. The diluted enzyme acts for 5 minutes at room temperature and pH 9.2 on a solution of arginine (4.5 per cent as the hydrochloride) in the presence of 0.125 M glycine and phosphate. After inactivating the enzyme with acid and expelling all CO_2, the pH is adjusted to 6.5 and urea decomposed with urease. From the CO_2 formed and the temperature of arginase action, the arginase concentration is determined. The total procedure is carried out in the Van Slyke-Neill blood-gas apparatus and requires 15 minutes.

Application of the gasometric ninhydrin-CO_2 method to determination of amino acids in blood. DONALD D. VAN SLYKE, DOUGLAS A. MACFADYEN and PAUL HAMILTON. *Hospital of the Rockefeller Inst. for Medical Research, New York City.* The method (D. D. Van Slyke and R. T. Dillon, Compt. rend. trav. Lab. Carlsberg **22**: 480, 1938; D. D. Van Slyke, R. T. Dillon, D. A. MacFadyen and P. Hamilton, J. Biol. Chem. **141**: 627, 1941) can be applied to blood or plasma at pH 2.5 without removal of proteins if a correction of 0.0005 of the protein nitrogen is subtracted. The amino acid determined includes about 5 per cent that has the properties of the lipo-amino acid, phosphatidylserine (J. Folch J. Biol. Chem. **139**: 973, 1941).

For routine analyses deproteinization is preferable. One volume of plasma is mixed with 5 volumes of 1 per cent picric acid. The analysis is run on 5 cc. of filtrate, with 100 mgm. of ninhydrin and 20 minutes heating. No buffer is added since the pH 1.8 of the filtrate is suited to the reaction. The mean difference between duplicates is less than 1 per cent.

Whole blood is analyzed in the same way, except that only 3 cc. of filtrate are required for the determination.

A slight amount of urea is hydrolysed by the heating, with evolution of CO_2 equivalent to 0.003 of the urea nitrogen. Except in uremic blood, this correction is ordinarily negligible.

Isolation of A 17-Ketosteroid sulfate. ELEANOR H. VENNING, M. M. HOFFMAN and J. S. L. BROWNE. *McGill Univ. Clinic, Royal Victoria Hospital, Montreal, Canada.* A crystalline conjugated 17-ketosteroid has been isolated from the urine of a man (case of Dr. Pierre Masson) with extensive metastases of an interstitial cell tumour of the testis. Approximately 1 gm. of 17-ketosteroid was excreted daily. A crude hydrolysed fraction gave a bioassay (kindly performed by Dr. F. C. Koch), based on the capon, of 12 to 18 per cent androsterone, or 47 to 57 per cent dehydroisoandrosterone.

The conjugated compound is water soluble, gives a violet color with the Zimmermann reagent corresponding to 77 per cent of dehydroisoandrosterone, and contains Na and a sulfate radicle. When crystallized from ethyl alcohol it melted sharply at 144°; when recrystallized from methyl alcohol it melted at 188–192° with decomposition. On simultaneous hydrolysis with hydrochloric acid and extraction with carbon tetrachloride considerable destruction occurred, only 40 per cent of the original ketosteroid content could be recovered. The free compound is ether soluble, gives a color with the Zimmermann reagent corresponding to 106 per cent in terms of dehydroisoandrosterone, and it has a melting point of 101–102–5°C. Another non-crystalline conjugated 17-ketosteroid has been isolated, M.P. 169°. It is also a sulfate.

Production of cholic acid by the rat. ROBERT W. VIRTUE. *Dept. of Chemistry, Univ. of Denver, Denver, Colo.* One group of rats was fed protein (codfish) for 3 days. A second group was fed fat (butter) for 3 days. A third group was given carbohydrate (sucrose and starch) for 3 days. All three foodstuffs were fed in equicaloric amounts per kilo body weight. Bile fistulas were then established in all the animals and the bile was collected in bulbs which were fastened outside the body. Bile was collected for 30 hours following each operation. The rats which had been fed protein secreted more cholic acid per kilo in the bile than did those fed either fat or carbohydrate.

The higher fatty aldehydes. HEINRICH WAELSCH

and MARJORIE ANCHEL. *Depts. of Neurology and Biochemistry, College of Physicians and Surgeons, Columbia Univ., New York City.* For the study of the metabolic fate of the higher fatty aldehydes methods for their isolation from brain tissue and muscle have been developed. From the alcoholic extracts of the tissue a lipid emulsion was prepared and was treated with dilute acid. The liberated aldehydes were coupled with p-carboxyphenylhydrazine or carboxymethoxylamine; derivatives corresponding to as much as 30 mg. of aldehydes per 100 grams of muscle tissue of cattle and rat were isolated. The carboxymethoximes from muscle and brain and the hydrazones from brain tissue were obtained in crystalline form. The analyses of the top fraction obtained on crystallization indicate the presence of aldehydes of average chain length of C_{17}.

It was found that other derivatives of the aldehydes could be prepared directly from the hydrazones by treatment with an excess of another aldehyde reagent, such as thiosemicarbazide.

The method described enables us to investigate the metabolism of the higher fatty aldehydes in laboratory animals. Experiments to determine the role of the aldehydes in lipid metabolism are being carried out with deuterium as an indicator.

Direct measurement of catalase activity with the dropping mercury electrode. BURNHAM S. WALKER. *Evans Memorial, Mass. Memorial Hospitals, and Boston Univ. School of Medicine, Boston.* Last year the author published a method for the amperometric measurement of catalase, in which the decrease in concentration of hydrogen peroxide was measured. The present method eliminates the use of solutions of hydrogen peroxide. This is done by utilizing the observation of Brdicka and Tropp, that in the presence of hemoglobin and other substances with catalase activity, the hydrogen peroxide component of the polarogram of oxygen is diminished or suppressed, with a corresponding increase in the oxygen component. The increase in current, at a stated voltage (e.g., -0.7 volt) intermediate to the half-wave potentials of oxygen and hydrogen peroxide, varies with the amount of catalytically active substance added (blood or crystalline catalase). The relationship between the current and the catalase activity, measured by titration, is not linear. Since the current-activity curve becomes asymptotic at a value of the reaction velocity constant of about 0.07 (obtained with about 0.0035 cc. of blood in 100 cc. of buffer solution), the method is adapted to the measurement of only very small amounts of catalase. The buffer solution of Strnak (0.1 N ammonium chloride in 1.6 N ammonia) is satisfactory for the polarographic measurement. A manual polarograph was used.

The rôle of pyruvate in the metabolism of ethyl alcohol. W. W. WESTERFELD, ELMER STOTZ and ROBERT BERG. *Dept. of Biological Chemistry, Harvard Medical School, Boston, and McLean Hospital, Waverly, Mass.* Oral administration of two 5 gm. doses of sodium pyruvate to dogs that had received 2 to 3 cc. of absolute alcohol per kgm. 4 to 6 hours earlier, increased the average rate of alcohol metabolism 260 per cent. The rate of decrease of the blood alcohol concentration changed from an average of 8.1 mgm. per cent per hour during the control period to 21.1 mgm. per cent per hour following the pyruvate. Alanine given orally in comparable doses exerted the same effect after a lag period of about one hour.

The feeding of 5 grams of sodium pyruvate to an untreated dog resulted in an increase in the venous blood pyruvate from 1.5 mgm. per cent to 4.5 mgm. per cent in 30 to 40 minutes and a return to normal in about 2 hours. When given to a dog that had received alcohol, pyruvate was utilized so rapidly that it produced only a slight increase in the blood pyruvate. Therefore pyruvate and alcohol disappeared simultaneously. Blood lactate rose to approximately the same level in the presence or absence of alcohol.

These results are interpreted as evidence that in the metabolism of alcohol, the acetaldehyde produced by the primary oxidation is condensed with pyruvate to form acetoin. This condensation (acetaldehyde and pyruvate) by an animal enzyme system was recently demonstrated in vitro by Green, *et al.* This metabolic pathway for alcohol furnishes an explanation for the well-known relationship between carbohydrate and alcohol metabolism, and for many other facts observed in the study of alcohol.

Effect of oral administration of aniline and *p*-aminodimethylaniline on the growth of the rat. JULIUS WHITE and JESSE E. EDWARDS. *National Cancer Inst., National Inst. of Health, U. S. Public Health Service, Bethesda, Md.* The growth-inhibiting action (failure to gain weight) of aniline and *p*-aminodimethylaniline has been demonstrated on young growing rats by the incorporation of either of these compounds into a basal diet of relatively low protein content. Thus the addition of either aniline (80 mgm.) or *p*-aminodimethylaniline (60 mgm.) to each 100 grams of the basal diet results in a change of the daily growth rate from an average of 1.2 grams on the basal diet alone to an average of 0.4 gram. The addition of either *l*-cystine (500 mgm.) or *dl*-methionine to each 100 grams containing either of the amines results in a prompt stimulation in growth, simulating the daily weight increments observed in rats ingesting the basal diet alone. Supplements of either taurine, glycine, or sodium

sulfate on the other hand did not stimulate growth when added to the diets containing either the aniline or *p*-aminodimethylaniline. Attempts are being made to determine whether these compounds produce cancer of the liver since they represent possible breakdown products of the cancer producing substance *p*-dimethylaminoazobenzene (butter yellow).

THE AMERICAN SOCIETY FOR PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS

THIRTY-FIFTH ANNUAL MEETING

Boston, Mass., March 31, April 1, 2, 3, 4, 1942

(For possible corrections in any of the following abstracts see the next issue)

An analysis of the blood pressure and repiratory changes produced by strychnine convulsions. B. E. ABREU and R. A. WOODBURY. *Dept. of Physiology and Pharmacology, Univ. of Georgia School of Medicine, Augusta.* Employing the "hypodermic" manometer, pressor and depressor responses after strychnine sulfate (0.1–0.2 mgm./kgm. intravenously) were investigated in unanesthetized dogs and cats. The usual increase in the arterial pressure during the tonic convulsion was 20–30 mm. Hg, and was produced by an increased intrathoracicoabdominal pressure, an increased venous return and an increased peripheral resistance resulting from the intense contraction of skeletal muscles. Immediately after each convulsion, pressor and/or depressor effects were observed. The contribution of the sympathetic and parasympathetic nervous systems and the skeletal muscular system to these blood pressure changes were estimated by using ergotoxin, atropine and beta-erythroidin, and by means of vagotomy and high spinal anesthesia with procaine.

A marked and prolonged decrease in blood pressure occurred immediately after each convulsion in vagotomized, ergotoxinized animals. This could be prevented by beta-erythroidin. In other experiments, after beta-erythroidin and vagotomy, cutaneous stimulation resulted in a marked and prolonged blood pressure increase, which subsequently was prevented by ergotoxin. Cutaneous stimulation after inactivation of the sympathetic nervous system and skeletal muscular system by spinal anesthesia resulted in an excessive cardiac slowing which could be abolished by atropine.

Cardiac arrest of vagal origin frequently followed convulsions, but only when aeration was inadequate. Convulsions during 45–60 minutes in adequately ventilated animals failed to paralyze the respiratory center. These same animals when inadequately ventilated showed Cheyne-Stokes respiration. However, subsequent adequate aeration effected a recovery of the respiratory center even when convulsive seizures continued.

This work was aided by a grant from the Josiah Macy Jr. Foundation.

Use of the Cope pouch dog for the study of the effect of gastric antacids on gastric secretion. W. LLOYD ADAMS, C. STUART WELCH and BYRON B. CLARK. *Depts. of Physiology and Pharmacology, and Surgery, Union Univ., Albany Medical College, Albany, N. Y.* The question of the so-called "rebound" of gastric acidity after the administration of gastric antacids is being studied by use of the Cope pouch dog in which vagus nerves are intact and collection vessels, bandages, and restraints are eliminated. A technic for maintaining electrolyte and water balance has been combined with a feeding regime which permits long-time 24 hour studies of gastric secretion. Data from such studies will be presented to show the effects of antacids introduced into the pouch or fed by mouth, and these will be discussed in relation to "rebound" secretion.

Absorption of sulfadiazine after intraperitoneal administration in dogs. A. M. AMBROSE. *Dept. of Pharmacology, Univ. of Louisville School of Medicine, Louisville, Ky.* In four dogs operated under sterile conditions powdered sulfadiazine, 100 mgm./kgm., was placed on the intestines directly beneath the greater omentum, the incision closed and the dogs permitted to recover. Absorption of the drug was followed hourly during the first 7 hours following administration and at 24 hour intervals thereafter.

The peak blood concentrations occurred 3 to 5 hours after administration and absorption of the drug continued for 144 hours. During the first 7 hours the average blood concentrations were—1.96, 3.0, 3.64, 3.5, 3.32 and 3.23 mgm./100 cc. re-

spectively; slightly less than that reported for sulfathiazole (Proc. Soc. Exp. Biol. and Med. 48: 223, 1941), about the same as for sulfaguanidine (Ambrose & Haag, unpublished data) and considerably less than for sulfanilamide (Haag, Spealman and McCue, Surgery, in press), using the same dose of each (100 mgm./kgm.) under similar conditions.

The sulfadiazine blood concentration after 24 hours averaged 2.4 mgm./100 cc. as compared to approximately 1 mgm./100 cc. for each of the other sulfonamides. The average sulfadiazine blood concentrations for each of the succeeding 24 hour periods were— 1.5, 0.72, 0.6, 0.5 and 0.39 mg./100 cc.

In two of the dogs urinary excretion of the drug was followed at 24 hour intervals over a period of 96 hours. During the first 24 hour period 37 per cent of the drug was excreted, in the second 7 per cent, in the third 5 per cent and in the fourth 4 per cent. A total of 53 per cent of the drug administered was absorbed and excreted within 96 hours after administration.

Assuming the bacteriostatic action of sulfadiazine to be equal to sulfanilamide, sulfathiazole, or sulfaguanidine these findings suggest that sulfadiazine is superior for local use because of its slower rate of absorption.

Absorption of sulfadiazine after intraperitoneal administration in man. A. M. Ambrose and R. Arnold Griswold. *Depts. of Pharmacology and Surgery, Univ. of Louisville School of Medicine, Louisville, Ky.* The action of the sulfonamides on microorganisms have been thoroughly investigated. As far as we have been able to determine no one had studied the rate of absorption of sulfadiazine after the intraperitoneal administration in man or the safety of reinforcing the local treatment by oral administration of sulfadiazine or other sulfonamides.

In four patients subjected to abdominal surgery 5 grams of powdered sulfadiazine was placed in the peritoneal cavity, absorption of the drug was followed every 2 hours during the first 24 hours after administration, every 6 hours for the next 24 hours, and every 12 hours there-after until the patient was either discharged from the hospital or the test was negative.

In the first patient the peak blood concentration of 3.17 mgm./100 cc. was reached in 108 hrs., in the second 3.83 mgm./100 cc. in 2 hrs., in the third 2.15 mgm./100 cc. in 3 hrs., and in the fourth 1.93 mgm./100 cc. in 6 hrs. after administration.

In the first patient 228 hrs. after administration the blood was free of sulfadiazine. Total urinary excretion was 98.9 per cent of the amount administered. The second patient showed a blood concentration of 0.43 mgm./100 cc. 444 hrs. after administration, with 99.3 per cent of the drug administered excreted in the urine, and the fourth patient showed a blood concentration of 0.77 mgm./100 cc. 453 hrs. after administration, with urinary excretion of approximately 50 per cent of the amount administered. In all 3 of these patients in which urinary excretion was followed, about 30 per cent of the drug was excreted in conjugated form the remainder being excreted in free form. Determination of free and total sulfadiazine in over 200 blood samples from patients reported here and others showed the absence of any conjugation in all but one blood sample. In the absence of more direct evidence this would suggest that sulfadiazine is conjugated in the kidneys.

The findings suggest that sulfadiazine is slowly absorbed from the abdominal cavity and that reinforcing the local treatment by oral administration of sulfadiazine or other sulfonamides, wherever indicated, may well be undertaken.

Anthelmintic action of certain halogenated hexyl and hexoyl resorcinols. Hamilton H. Anderson and Peter P. T. Sah. *Peiping Union Medical College.* An objectionable feature of hexylresorcinol is its locally irritating effects on mucous membranes. Ten halogenated hexyl and hexoyl resorcinols have been prepared and studied for anthelmintic effectiveness by the method of Lamson and Brown (Am. J. Hyg. 23: 85, 1936). The physical properties of the compounds, the time in minutes required to kill *Ascaris lumbriccoides in vitro* on exposure to 0.1 per cent concentration, and the degree of local irritation of the buccal mucosa of human subjects follow: 6-n-hexyl, MP 68–69°C., 3, marked; 6-n-hexoyl, BP 185–190°C. at 2 mm., 6, marked and persistent; 4-chloro-6-n-hexyl, BP 120–122°C. at 4 mm., 6, moderate and transient; 4-chloro-6-n-hexoyl, BP 185°C. at 4 mm., 9, slight and transient; 2,4-dichloro-6-n-hexyl, MP 40–42°C., 9, slight and transient; 2,4-dichloro-6-n-hexoyl, MP 101–102°C., 120, none; 4-bromo-6-n-hexyl, BP 11–14, 21, moderate and transient; 4-bromo-6-n-hexoyl, MP 29–30°C., 25, moderate and transient; 2,4-dibromo-6-n-hexyl, MP 37–39°C., 26, none; 2,4-dibromo-6-n-hexyl, MP 23–27°C., 22, marked and persistent; 2-bromo-4-chloro-6-n-hexyl, BP 155–156°C. at 14 mm., 35, marked but subjective only; 2-bromo-4-chloro-6-n-hexoyl, BP 170–175°C. at 6 mm., 25, marked and transient. Local irritant action roughly parallels anthelmintic power in this series, with the exception of di-bromo-hexylresorcinol, which is an effective anthelmintic *in vitro* and yet is not locally irritating to the buccal mucosa of man.

The development of tolerance to demerol. H. L. Andrews. *U. S. P. H. S. Hospital, Lexington, Ky.* Demerol (1-methyl-4-phenyl-piperidine-4-carbonic acid ethyl ester) was administered regularly to four patients. Using the Hardy-Wolff

method determinations of the pain threshold raising effect were made at weekly intervals during the period of administration and at 15-day intervals following withdrawal. In each case there was a reduction in the pain threshold raising effect of the drug as the study progressed. At the end of eight weeks tolerance appeared to have reached a maximum. Tolerance is maintained for at least 30 days after withdrawal. This effect appears to be similar to but perhaps less permanent than the tolerance developed to morphine.

The effects of various concentrations of carbon dioxide on the toxicity of ethyl (1-methyl butyl) barbituric acid. J. H. BARBOUR and M. H. SEEVERS. *Dept. of Pharmacology, University of Wisconsin, Madison.* An anesthetic dose (40 mgm. per kgm.) of sodium ethyl (1-methyl butyl) barbituric acid was administered intraperitoneally to each of eighty albino rats. One group of twenty animals served as control, and three other groups of twenty each were placed in 5, 10 and 20 per cent carbon dioxide, respectively. The mean recovery time was 5 hours in both the control group and those exposed to 5 per cent CO_2; 7 hours in 10 per cent CO_2, and 13 hours in 20 per cent CO_2. Repetition of this experiment produced similar results. Such concentrations of CO_2 alone produce only a slight grade of depression although it is clear from these experiments that a significant additive narcotic action is demonstrable in the anesthetized animal.

Other experiments were performed in an identical manner except that the dose of the barbiturate represented the accepted $M.L.D._{.50}$ (75 mgm. per kgm.). In these experiments, as in the one described above, the recovery time was prolonged greatly in those animals exposed to 10 and 20 per cent CO_2. However, no additive toxicity was demonstrable with respect to the lethal dose. On the contrary, the mortality was actually fifty per cent less in the groups exposed to 5 and 10 per cent CO_2, due presumably to better respiratory activity.

The results of still other experiments suggest that the prolongation of recovery time in the presence of CO_2 may be related to a decreased rate of destruction of the barbiturate although this has not been proven definitely to be the case.

Ejaculation induced by a uterine drug. GEORG BARKAN. *Evans Memorial, Mass. Memorial Hospitals and Boston Univ. School of Medicine.* The di-ethyl-amino-ethyl-ether of 2-methoxy-6-allyl-phenol in form of its hydrochloride introduced as a uterine drug (Gravitol) was pharmacologically and toxicologically investigated in previous work by G. Barkan and associates. In further study it has been found that the drug when applied subcutaneously in male guinea pigs has a prompt ejaculatory effect with or without coagulation of the ejaculate in situ and with living spermatozoa visible under the microscope. The minimum effective dose in this respect was found to be 0.02 gram of the drug per kilogram body weight or between 50 and 60 per cent of the minimum lethal dose. The effect occurs 10–50 minutes after the application and can be reproduced repeatedly in the same guinea pig on different days. Intragastric administration of the tenfold dose had the same effect.

The effectiveness in man of demerol, a new analgesic drug. ROBERT C. BATTERMAN. *New York Univ. College of Medicine.* Demerol (1-methyl 4-phenyl piperidine 4-carbonic acid ethyl ester hydrochloride), a new synthetic analgesic, was found to be a satisfactory and safe drug in over 800 patients, most of whom ordinarily would have required opiates for relief. The dose is 50 to 150 mgm. orally or intramuscularly, in a single dose or repeated doses several times daily. An effect is apparent within 20 to 60 minutes orally or 15 minutes parenterally. In either case the effect lasts from one to several hours with an average duration of 2 to 3 hours. Postoperative patients, regardless of surgical procedure, in the majority of instances are maintained comfortably without difficulty. Pains of visceral origin are effectively relieved (gastro-intestinal intubation studies in man with balloons have demonstrated an antispasmodic action contributing to the analgesic effect). Arthritic, neuritic and vascular pains are also relieved. Nausea and vomiting occur in approximately 5 per cent of the cases. Especially in ambulatory patients other side effects, which are of short duration and usually insignificant, are noted. These are dizziness, perspiration, lightheadedness, dryness of the mouth, and euphoria. Extreme weakness with marked dizziness and vomiting occurs rarely. Drowsiness and sleep occur with the larger doses, usually following parenteral administration, are of short duration, and are not followed by depression or confusion. No toxicity in the hematopoetic, genito-urinary, respiratory, or cardiovascular systems, and no evidence of addiction following prolonged use have been noted.

Preliminary studies on the pharmacology of dl-β-phenyl-n-propylmethylamine. T. J. BECKER, M. R. WARREN, D. G. MARSH, C. R. THOMPSON and R. S. SHELTON. *Research Labs., The Wm. S. Merrell Co.* dl-β-Phenyl-n-propylmethylamine, a secondary amine, is a colorless liquid having a characteristic odor. It has a molecular weight of 149.1 and a boiling point of 211°C. On exposure to air it forms a carbonate from which the amine readily volatilizes. For pharmacological study the hydrochloride salt of this compound was employed.

The acute as well as the chronic toxicity of dl-β-phenyl-n-propyl-methylamine has been deter-

mined following both oral and intravenous administration. The approximate M.L.D., when administered intravenously, is 60 mgm. per kgm. and 500 mgm. per kgm. when administered orally.

Preliminary studies indicate that this compound in a dose of 1 mgm. per kgm. produces a rise in blood pressure and a peripheral vasoconstriction equal to that elicited by similar amounts of l-ephedrine or dl-β-phenylisopropylamine. Its action on the bronchioles appears to be less than that of l-ephedrine.

dl-β-Phenyl-n-propylmethylamine in a concentration of 1:5,000 usually relaxes the isolated jejunum of rabbits. At times this action is very slight. The isolated rabbit uterus is always stimulated at this same concentration.

Results of experiments on rats indicate that dl-β-phenyl-n-propylmethylamine produces very little central stimulation. These results are compared with those obtained with similar amounts of l-ephedrine and dl-β-phenylisopropylamine.

The effect of multiple mosquito bites on the course of Plasmodium cathemerium infection in canaries. HARRY BECKMAN. *Dept. of Pharmacology, Marquette Univ. School of Medicine, Milwaukee, Wis.* One hundred and fifty-four canaries were bitten by *Culex pipiens* mosquitoes carrying the sporozoites of *P. cathemerium:* 58 by 1 mosquito, 66 by 2 mosquitoes, and 30 by 3 mosquitoes. Analysis of the resultant infections shows that multiple-bite infections are superior to single-bite infections since they produce a higher rate of successful takes, a shorter incubation period, and possibly slightly more severe infections (shorter duration of attack in fatal cases). Nevertheless, evidence is deduced from the experiment to indicate that it will probably be more satisfactory to develop the standardized infection upon the basis of a bird bitten by only one mosquito.

A heated thermocouple flow-meter for recording flow in blood vessels of experimental animals. H. S. BENNETT and W. H. SWEET. *Depts. of Pharmacology and Anatomy, Harvard Medical School, Boston, Mass.* (Demonstration). A heated iron-constantan thermocouple is cemented into a machined lucite cannula. The wires are very fine (0.0015″) and are covered with a thin layer of insulating plastic. Both junctions project into the lumen of the cannula, which is inserted into the two cut ends of a severed blood vessel. The downstream junction is heated by passing a constant current through a constantan wire soldered to the junction. The upstream junction remains at blood temperature. The current from the thermal junctions is passed through a moving-coil galvanometer, whose deflections are recorded optically. The galvanometer circuit is provided with a counter-current, which can be quickly and conveniently calibrated. Leads to the junctions in the cannula and to the galvanometer are shielded; all resistances in the galvanometer circuit are of manganin or other low temperature coefficient wire; all permanent connections are soldered; sealed mercury switches are used in the galvanometer circuit; low contact resistance plugs are used where connections must necessarily be interchangeable. Each cannula is individually calibrated with citrated beef blood at 37.5°C. Calibrations are repeatable from day to day, within a small experimental error. The animal must be heparinized.

The use of very fine wires projecting directly into the blood passing through a cannula of constant diameter allows one to record flow changes continuously with less lag and more accuracy than can be achieved with flow-meters previously described in which the junctions are exterior to the blood vessel.

The convulsive action of the commonly used local anesthetics. R. BEUTNER and B. CALESNICK. *Dept. of Pharmacology, Hahnemann Medical College, Philadelphia, Pa.* In order to determine the relation between local anesthetic efficiency and convulsive action, 14 widely used local anesthetics were injected in guinea pigs intramuscularly in varying concentrations and the minimal dose determined which led to convulsions in 90 to 100 per cent of the animals. This dose is not always identical with the fatal dose. The anesthetic potency of the same drugs was determined through the minimal concentration which would produce a superficial anesthesia on the eye of rabbits for only 10 minutes. This concentration was 1:5000 for pontocaïne, about 1:1000 for cocaine, butyn and others; for procaine only about 1:30. In contrast; the ratio of convulsive dose to anesthetic concentration varies only from 30,000 to 50,000 for most penetrating local anesthetics, about 6,000 for procaine and other non-penetrating anesthetics. A nearly parallel course of convulsive action and local anesthetic power is thus revealed. Drugs which are relatively less convulsant than corresponds to their anesthetic potency, as e.g. diothane, appear to be extraordinarily irritant, hence their action is probably partly due to tissue damage.

Chemically, all these soluble local anesthetics are esters of amino alcohols. Non-esters, like benzyl alcohol and quinine-ures-HCl, which are also non-convulsant, appear to be devoid of local anesthetic potency proper and to act only by irritation, as indicated by experiments which will be discussed in detail. Nupercaine, the strongest of all introduced local anesthetics, is in a class by itself since its fatal and convulsive doses coincide, and for other reasons to be explained.

The local action of mercurial diuretics. R. BEUT-

NER and B. CALESNICK. *Dept. of Pharmacology, Hahnemann Medical College, Philadelphia, Pa.* Continuing the experiments of Beutner, Landay and Liebermann new observations were made which indicate that salyrgan has a local tissue action. It retards or inhibits the resorption of an intramuscularly injected drug or dye when it is added to that drug or dye solution and injected simultaneously with it. The retarded absorption of many convulsive poisons like strychnine, picrotoxin, procaine is demonstrated by the inhibition of the onset of convulsions by otherwise convulsive doses if salyrgan is added to them. If salyrgan is injected separately it fails to check convulsions. Likewise the resorption of a dye like phenol red into the blood stream is retarded after intramuscular injection when salyrgan is added. This inhibition of resorption cannot be due to direct combination of the convulsant drug or dye with salyrgan, as assumed by Haury, since the diffusion of these drugs through parchment shells is not at all inhibited by salyrgan. Moreover, it would be hard to understand how salyrgan can combine with both basic substances (like strychnine or procaine) and also with picrotoxin or phenol red which have an acid character. Mercurin, another mercurial diuretic, was also found to inhibit resorption but novasurol showed no effect of this type. Xanthin diuretics not only fail to inhibit but actually counteract the inhibitory effect of salyrgan or mercurin. It may be assumed that the inhibition of resorption of the type described is the result of a setting free of water by cells or tissue membranes as indicated by well-known biological observations of R. S. Lillie and others.

The pharmacological properties of ortho-nitrophenol. R. BEUTNER and A. COHEN *Dept. of Pharmacology, Hahnemann Medical College, Philadelphia, Pa.* Our previous investigations showed that o-nitrophenol is more resistant to chemical changes in the body metabolism than other phenol derivatives. Excretions tests now showed that after intramuscular injection in rabbits σ-nitrophenol can be recovered as completely from the urine as seems possible. If anointed on the human skin in a suitable ointment, about 5 per cent of the total nitrophenol appears in the urine. After a very extensive inunction the total nitrophenol resorbed is only about 1/1000 of the tolerated dose in animals. This tolerated dose was determined in guinea pigs, rabbits, mice and dogs. All these animals stood doses of 500 mgm./kgm. without any apparent symptoms, mice even higher doses. (Cats proved to be more sensitive, no more than 100 mgm./kgm. being tolerated.) In contrast, the highest amount of nitrophenol which might be resorbed from a cutaneous anointment is as low as 0.1 to 0.5 mgm./kgm.!

Dinitrophenol is known to have lead to cataract formation after prolonged internal use. This late chronic poisoning is probably related to its calorigenic action and disturbance of metabolism. In contrast, o-nitrophenol has *no* calorigenic action at all as determined by direct metabolic tests; 100 mgm./kgm. injected in oil solution in rats proved to be without any perceptible metabolic effect. (Extensive use of nitrophenol ointment in hundreds of human cases never showed a single cataract after years.) Moreover, chronic toxicity tests showed that the drug has no demonstrable effect upon internal organs after repeated injections in rabbits over prolonged periods of time.

The effect of sulphonamides upon peptone fever. ELDON M. BOYD. *Dept. Pharmacology, Queen's Univ., Kingston, Canada.* The antipyretic action of sulphonamides was studied by investigating their effect upon artificial fever induced by the parenteral injection of peptone into albino rats, cats and rabbits. The sulphonamides were given by stomach tube in doses of 0.1 grams per kilo body weight two hours after the injection of peptone and rectal temperatures were recorded every half hour. Sulphanilamide had a pyretic effect upon normal body temperature in cats and rabbits but did not influence peptone febrile temperatures in cats, rabbits or albino rats. Sulphapyridine was antipyretic toward peptone fever. Sulphathiazole had no effect upon normal or upon peptone febrile temperatures. Sulphaguanidine and sulphadiazine were likewise without effect upon normal or artificial febrile temperatures. Additional studies were made upon sulphapyrazine, azosulphamide, benzylsulphanilamide, sulphones, sulphanilylacetamide, succinylsulphanilamide, and sulphanilyl-dimethyl-sulphanilamide.

Experimental and clinical studies on the effect of methyl-isopropyl-phenol on tuberculosis. CLYDE BROOKS and HARVEY B. SEARCY. *School of Medicine, Louisiana State Univ., New Orleans.* A series of guinea pigs were inoculated with virulent tubercle bacilli.

After two weeks the animals were divided into two equal groups. One half were used for controls; the other half were treated by daily oral administration of methyl-isopropyl-phenol. After some weeks the treated animals are all living, while several of the controls are dead. The end results will be reported in detail.

A series of human cases of tuberculosis of the lungs were selected. Only advanced cases who were not improving under hospitalization and bed rest, and other forms of treatment, were accepted. These patients were treated with daily oral administration of methyl-isopropyl-phenol.

Another series of cases similar in character were observed for controls.

After several weeks the treated cases are gen-

erally improving, while the controls are not. Several deaths have occurred in the controls, and no deaths in the treated cases. Results up to date will be reported in detail.

Toxicity and antiseptic action of cresylic acids. JOSEPHINE CAMPBELL. *Univ. of California Medical School.* Mixtures of alkyl phenols (cresylic acids) are obtained from petroleum with higher boiling points than those obtained from coal tar. Pharmacological comparisons of these two types of cresylic acid mixtures show that 1, an hour's inhalation of air saturated with the vapors of either causes local irritation of eyes and upper respiratory tract and similar exposures repeated daily may produce several reactions; 2, high boiling petroleum alkyl phenol mixtures are less toxic on oral administration and less irritating to the skin than low boiling coal rat cresylic materials, although both types may be absorbed through the skin with toxic symptoms; 3, disinfectants prepared from high boiling petroleum cresylic acids in a manner similar to saponified solution of cresol USP XI, or Commercial Standards Specifications CS 70-38 and CS 71-38, are bactericidal at 20 minutes application to standard cultures of *S. aureus* and *B. coli* at a concentration of 0.05 per cent, without inhibition of embryonic chick heart on 24 hours application, while saponified solution of cresol USP XI and similar disinfectants made from coal tar cresylic acids reduce growth and activity of embryonic chick heart and are not bactericidal at these time-concentrations.

The influence of ouabain on the "effciency" and force of contraction of striated muscle. MCKEEN CATTELL. *Dept. of Pharmacology, Cornell Univ. Medical College, New York City.* The digitalis glucosides act directly on cardiac muscle to increase the force of contraction, and this occurs, according to Visscher et al., without a corresponding increase in oxygen consumption. In both cardiac and striated muscle digitalis causes the cell to lose potassium and it becomes of interest to determine whether both undergo the same changes in the energetics of contraction.

Using the myothermic technic of A. V. Hill, we have measured the initial heat production and the tension of the twitch of the sartorius muscle of the frog before and after exposure to weak solutions of ouabain. After several hours in oxygenated Ringer's solution the tension/heat ratio usually declined, and in one group of seven experiments fell to 70 per cent of the original value in the course of 18 to 24 hours. After exposure to ouabain-Ringer's solution (1 part in 100 or 200 thousand) the tension/heat ratio recovered to 87 per cent of its original value. The tension of the twitch, after passing through a stage of augmentation, dropped to 62 per cent of the initial value, and after ouabain recovered to 78 per cent.

In freshly isolated muscles and in muscles from certain batches of frogs which, perhaps due to seasonal differences, did not exhibit a drop in the efficiency of contraction over a 24-hour period, the effects of ouabain were slight or entirely absent.

Thus ouabain acts on striated muscle as on cardiac muscle to increase the force and improve the efficiency of contraction when function is depressed.

Larvicidal action of chloro-hexyl-meta-cresol. Y. C. CHIN and HAMILTON H. ANDERSON. *Peiping Union Medical College.* It has been shown (Peking Nat. Hist. Bull. **16**: 45, 1941) that chloro-hexyl-meta-cresol is the most active of 18 related compounds in killing adult *Drosophila melanogaster.* This substance is comparatively non-toxic. Its LD_{50} for rats on gastric administration is 6.0 cc. per kgm., while for mice it is 4.0 cc. per kgm. on subcutaneous injection. We have now compared the larvicidal action of the hexyl-cresol isomers and their monochlorinated derivatives against 3 species of mosquitoes, *Anopheles hyrcanus, Culex pipiens, Aedes koreicus.* One-half per cent soap suspension has no larvicidal effect. Using 1 per cent pyrethrum in 0.5 per cent soap suspension as a potent larvicidal standard, killing from 94 to 100 per cent of all larvae after 30 minutes exposure, we found that chloro-hexyl-meta-cresol in 0.2 per cent concentration in 0.5 per cent soap suspension is its equal or superior. Chloro-hexyl-ortho-cresol kills about 90 per cent of all larvae under such conditions.

The activity of ya-tan-tzu as an amebacide in vitro. T. K. CH'UAN. *Peiping Union Medical College.* Ya-tan-tzu, which is probably *Brucea javanica,* has been reported to have some value as an anti-amebic drug (Chin. Med. J. **60**: 229, 1941). Since clinical results have been encouraging it was believed desirable to determine the effect of aqueous infusions of oil-free portions of the kernels. Specimens obtained from Kunming, Canton and Peiping were powdered and prepared by repeatedly washing the oil-free powder with sterile distilled water. The aqueous suspension was filtered and neutralized with $NaHCO_3$ and mixed with an equal volume of 1.8 per cent NaCl solution.

Three strains of *E. histolytica,* two isolated from man and one from *Macaques rhesus,* were grown on Dobell's coagulated egg medium at 37°C. The effect of Ya-tan-tzu in various dilutions was observed at 24 and 48 hours. In 1:1000 dilution, 2 of the 3 strains of amebas were killed after 48 hours exposure. This result would indicate that the direct amebacidal action of the 3 specimens of Ya-tan-tzu tested was insufficient to account for the reported clinical value of the drug.

The acute effects of hypoxia resulting from methe-

moglobin produced by aniline. BYRON B. CLARK and EDWARD J. VAN LOON. *Dept. of Physiology and Pharmacology, Union Univ., Albany Medical College, Albany, N. Y.* The *in vivo* production of MHb (methemoglobin) requires administration of chemical agents which also have other pharmacological actions. Aniline was chosen because small doses (10 to 100 mgm. per kgm., *per os*) produced a relatively intense and prolonged methemoglobinemia, and various experiments have indicated that the effects of such doses were predominately due to MHb.

Aniline was administered *per os* to unanesthetized dogs. On behavior, MHb concentrations up to 45 per cent produced no observable change; 45 to 55 per cent caused a slight decrease in spontaneous activity and slight lethargy; 55 to 60 per cent caused decreased activity, increased lethargy and some ataxia; 60 to 75 per cent caused progressively, confusion, gross ataxia, inability to stand, followed by a semicomatose state in which there later appeared extensor spasms, unprovoked motor restlessness, and exaggerated reflexes. Maintained concentrations of 70 to 75 per cent MHb resulted in death in 10 to 20 hours. These effects are considered to be due primarily to the hypoxia of the central nervous system.

Blood pressure was not immediately influenced by MHb concentrations up to 50 per cent. However, higher concentrations, if maintained, caused a slow progressive fall. MHb levels of 50 per cent or more always caused a sinus tachycardia. Further investigations are being made on the circulatory adaptation to this type of hypoxia which is apparently different from that produced by breathing low oxygen mixtures.

MHb concentrations up to 50 per cent do not produce an increase in pulmonary ventilation since arterial O_2 saturation (and presumably arterial pO_2) remains essentially normal.

The effects of a diet deficient in vitamin B on the toxicity of sulfapyridine. VERSA V. COLE and BEN KING HARNED. *Laby. of Pharmacology and toxicology, Woman's Medical College of Pennsylvania, Philadelphia.* Experiments on 93 rats have demonstrated that a deficiency of vitamin B in the diet increased the toxicity of sulfapyridine. The animals were matched according to sex and age and divided equally into the following groups; *a*, Goldberger-diet; *b*, Goldberger-diet plus sulfapyridine; *c*, Purina-diet plus sulfapyridine. The food was administered *ad libitum*. The Goldberger-diet consisted of white corn meal, casein extracted with 60 per cent alcohol, sucrose, cotton seed oil, cod liver oil, and Osborne and Mendel's salt mixture. The sulfapyridine was administered by stomach tube once or twice daily in doses ranging from 250 to 750 mgm./kgm./day for periods of 3 to 21 days.

Employing as criteria the prothrombin clotting time, the colloidal gold test for liver function, the percentage of acetylsulfapyridine excreted, the hematuria, the uroliths, the hydronephrosis and the death rate, the results show that the Goldberger-diet increased the toxicity of sulfapyridine.

A study of the relationship between anesthetic potency and physical properties. NELLIE M. CONE and C. JELLEFF CARR. *Dept. of Pharmacology, School of Medicine, University of Maryland.* A new series of cyclopropyl aliphatic ethers has been synthesized in this laboratory. The first two members of this series, cyclopropyl methyl ether (cyprome ether) and cyclopropyl ethyl ether (cypreth ether) have been shown to be useful general anesthetics. In comparative studies with these new agents it became necessary to study the physical properties of these compounds to determine their value in predicting anesthetic potency. The literature available on the oil/water coefficients of the generally used anesthetics is confusing and data are not concordant. The authors believe that this is due to: first, the technical difficulties inherent to the determination; second, the use of oils containing free fatty acids, and third, the employing of analytical procedures which lack precision. Accordingly, the field was restudied and more dependable comparative data obtained.

Ethyl ether, cyprome ether, cypreth ether, allyl methyl ether, chloroform, vinyl ether, ethyl-n-propyl ether and cyprethylene ether were studied. In this series of compounds we have found the simple determination of water solubility of the anesthetic to bear a striking relationship to the biologic potency. This datum appears to be a more faithful physical index than the oil/water coefficient and the simplicity of the determination bespeaks its general applicability.

Some effects of depressants of the central nervous system on the nembutalized cat. HELEN C. COOMBS and F. H. PIKE. *Brooklyn College and Columbia Univ. New York City.* It has recently been stated (H. K. Beecher and C. A. Moyer. Science **94**: Nov. 14, 1941; Science Supplement, P. 10) that the action of the barbiturates diminishes the sensibility of the central respiratory mechanism to the stimulus of carbon dioxide. It has been the frequent observation of both authors that in cats under nembutal, weak excitation of the central end of the vagus nerve results in death. It was pointed out by Coombs in 1939 (H. C. Coombs. Bull. N. Y. Medical College and Flower Hospital **2**: p. 1) that in nembutalized animals under conditions of cerebral anaemia, the cells of the central cardiovascular mechanism appear to survive longer than those which act to initiate respiratory movements. Moreover, in cats which have been heavily bromidized, prior to excision of the thy-

roids, great care has to be taken if they are nembutalized for the operation, and, in fact, the mortality is so high that we have found ether much safer. For interval adrenalectomies in bromidized cats, half the ordinary dose was all that could be tolerated for the second operation. This dosage (12 to 20 mgm. per kilo) was quite as effective as double the amount for the normal cat, and even then there was a prolonged period before the passing of the anaesthesia. Animals sometimes die from eight to twelve hours after the operation without regaining consciousness. This does not occur in etherized animals. We offer these data as confirmatory evidence of the more recent work of Beecher and Moyer.

Studies on the tolerance formation to gold salts in rats. RUTH CORTELL and R. KOHN RICHARDS. *Abbott Laboratories, North Chicago, Ill.* The minimum lethal dose of Gold Sodium Thiosulphate (g.s.th.) intramuscularly in rats was found to be 35 mgm. per kgm. Repeated injections of this dose resulted in a tolerance in the animals towards very much larger doses of g.s.th., such that these rats survived 200 per cent of the usually fatal dose. The development of tolerance was found to depend on the size and number of doses.

The tolerance to g.s.th. was found to persist in rats subjected to partial hepatectomy.

Since gold in the form of g.s.th. is excreted mainly by the kidney, careful histological studies of the kidneys were performed to determine any correlation between kidney changes and tolerance formation. It was found that severe tubular damage resulted from the first injection of 35 mg. per kg. of g.s.th., and as the repeated injections were given, repair of the renal epithelial tissue occurred. When the test dose of 100 mgm. per kg. was given to a tolerant rat, renal damage was very slight, whereas when the same dose was given to a normal rat, marked epithelial damage resulted.

The antispasmodic action of a series of new derivatives of isoquinoline. RAYMOND W. CUNNINGHAM and EDWIN J. FELLOWS. *Temple Univ., School of Medicine.* The spasmolytic activity of a series of compounds with papaverine-like nuclei, namely, 1-methyl, 1-phenyl, 1-benzyl and 1-phenethyl derivatives of 3-methyl-3,4-dihydro isoquinoline and 3 methyl-1,2,3,4-tetrahydro isoquinoline was determined and compared with that of papaverine. Five of the seven compounds relaxed smooth muscle spasm induced by agents with parasympathetic type of effect or by barium chloride. These derivatives were found to be much less toxic in mice than papaverine.

Placental transmission of blood iodine. NORMAN A. DAVID, NILKANTH PHATAK and ROSA KUBIN. *Univ. of Oregon Medical School.* We have reported that following organic iodide medication (Lipo-iodine-Ciba) there is a marked rise of maternal blood iodine, but the fetal (cord) blood iodine remains within the normal adult range. It has also been reported (G. E. Hudson, 1931) that after inorganic iodide medication, the maternal supply of ingested iodide reaches the fetal thyroid through placental circulation.

Determinations of several normal constituents of maternal (M) and fetal (F) blood made simultaneously with iodine showed that blood iodine (M. 14.4; F. 9.3 gamma per cent) and cholesterol (M. 225; F. 164 mgm./per cent), unlike blood chloride (M. 499; F. 481 mgm./per cent) and urea nitrogen (M. 8.3; F. 7.8 mgm./per cent) were at higher levels in the maternal than in the fetal blood in eighteen untreated cases. It has been suggested that circulating blood iodine may be partially combined with blood proteins or fragmented parts of serum proteins (Silver, S. 1940). Our results further indicate the possibility that the increase of maternal blood iodine after organic iodide medication may be associated with the increased lipid concentration of maternal blood. [*Supported by a grant from Ciba Pharmaceutical Co.*]

The action of unsaturated lactones on the isolated frog heart. ENRIQUE MOISSET DE ESPANÉS. *Dept. of Pharmacology, Harvard Medical School, Boston, Mass.* In recent studies from this laboratory it was found that simple unsaturated lactones like $\alpha\beta$-angelicalactone and $\beta\gamma$-angelicalactone have characteristic effects upon the frog heart. They cause systolic standstill and they counteract the effect of a salt solution poor in calcium ions.

These studies were extended to a group of other lactones. Particular attention was given to $\beta\gamma$-homoangelicalactone. Given once into the Straub heart in a concentration of 1:50,000, $\beta\gamma$-homoangelicalactone increases the amplitude of contraction. If the perfusion fluid is continuously replaced at a rate of 2–2.5 cc. per minute, systolic standstill is produced. The limit concentration of $\beta\gamma$-homoangelicalactone which produces systolic standstill is 1:500,000 to 1:1 million. Its potency is similar to that of $\beta\gamma$-angelicalactone.

The influence of temperature upon the systolic action of the angelicalactones is being studied.

The unsaturated lactones used in these studies were synthesized by R. P. Linstead, Converse Laboratory, Harvard University, Cambridge.

Toxic effects upon rabbits and cats of pentachlorophenol. WILHELM DEICHMANN and KARL V. KITZMILLER. *Kettering Laby. of Applied Physiology, Medical College, Univ. of Cincinnati, O.* Pentachlorophenol has come to have commercial importance as an agent for the control of microorganisms, and its uses include the preservation of wood and wood products, pulp and paper, tex-

tiles, starches, gums, glues, casein, albumen, leather, etc.

When pentachlorophenol is absorbed in sufficient quantity, it produces an acute toxemia characterized by accelerated respiration, hyperpyrexia, hyperglycemia and glycosuria, and by a rapidly developing motor weakness, which in fatal cases terminates in cardiac and muscular collapse. Rigor mortis is immediate and profound in most cases. The compound is rapidly absorbed from the stomach and through the skin and enters practically all organs and tissues, but most of the pentachlorophenol is always found in urine and blood. Skin, liver and muscle also contain large amounts; kidneys and bone usually contain less while heart, lungs, testes, brain cord, feces and the exhaled air contain mere traces.

The gastro-intestinal absorption of lanatoside C. JAMES M. DILLE and GEORGE B. WHATMORE. *Dept. of Pharmacology, College of Pharmacy, Univ. of Washington, Seattle.* The rate of absorption of Lanatoside C from the stomach, intestine and colon of cats was determined and compared to the rate of absorption of Tincture of Digitalis USP XI from the same sites. The drug was placed in the ligated portion of the gastro-intestinal tract after anesthetization with sodium pentobarbital. After one hour the ligated section was removed and the lethal dose determined by intravenous infusion. This value subtracted from a control value, obtained by determining the lethal dose under the same conditions of anesthesia and surgical procedures, gives the amount of cardio active glycosides absorbed in the time interval. The results obtained by this procedure, which is termed the determination of essential absorption, were compared to the results obtained by biologically assaying the residue in the excised section of the gastro-intestinal tract for cardio active glycosides. Absorption took place most rapidly from the intestine but quantities were also absorbed from the colon and stomach. Lanatoside C was absorbed more rapidly than the Tincture of Digitalis. Results also indicate that there is a likelihood of partial destruction of the cardio active principles in the gastro-intestinal tract.

The effect of ingestion of glycine and ascorbic acid on the toluene level in blood, the excretion of hippuric acid, and the blood picture of dogs exposed to various concentrations of toluene. D. D. DONAHUE, J. L. SVIRBELY, P. J. VALAER and W. F. VON OETTINGEN. *Division of Industrial Hygiene, National Inst. of Health, Bethesda, Md.* Dogs fed normal diet and diets supplemented with glycine or ascorbic acid were exposed to toluene vapor in concentrations of 200 to 600 p.p.m. Absorption, elimination and the effect of toluene on the blood picture were studied.

The toluene concentration in the blood after 7 hours' exposure was proportional to its concentration in the air, the toluene concentration in the blood of the glycine dogs being the lowest. Elimination from the blood was complete in 16 hours. Toluene elimination was most rapid in the dogs fed glycine.

Successive daily exposures to a toluene concentration of 400 p.p.m. did not affect the total leucocyte count, erythrocyte count or hemoglobin content of the blood. At the end of each daily exposure a slight percentile increase of lymphocytes with a corresponding decrease in neutrophils occurred. Neither the toluene level in the blood nor the hippuric acid excretion gave evidence of cumulative action.

Exposure of anesthetized dogs for one hour to toluene vapors of a concentration of 850 p.p.m. showed no significant changes in the arterial, venous or spinal pressures. The respiratory rate and volume increased rapidly during the exposure period; afterwards it fell gradually but remained somewhat elevated. The concentration of toluene in the blood and expired air decreased very rapidly during the first hour after exposure; later slowly.

Heparin as an antidote to trypsin in the rat. CARL A. DRAGSTEDT, J. A. WELLS and M. ROCHA E SILVA. *Dept. of Physiology and Pharmacology, Northwestern Univ. Medical School, Chicago.* The intravenous injection of an adequate dose of a commercial trypsin preparation (Trypsin-Difco) into lightly etherized rats is rapidly fatal. In a series of 49 rats the LD1, LD50 and LD99 were found to be 90, 137 and 250 mgm. per kilo respectively. The prior intravenous injection of heparin (20 to 40 mgm. per rat) protected animals completely against the LD99 and no deaths occurred until the dose of trypsin exceeded 300 mgm. per kilo.

The antitryptic action of heparin on the digestion of casein and on the coagulation of the blood has been noted by other workers. The present experiments support the view that the toxic properties of trypsin are related to its enzymatic action. (*This investigation has been made with the assistance of a grant from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association, and a grant from the Clara A. Abbott Fund of Northwestern University.*)

The percutaneous absorption of ammonium hydrogen sulfide and hydrogen sulfide. J. H. DRAIZE and E. P. LAUG. *Division of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D. C.* When a solution of ammonium hydrogen sulfide is applied to the skin of animals, penetration occurs as is shown by 1, a syndrome of respiratory effects leading to complete respiratory failure; 2, excretion of hydrogen sulfide by the lung, and 3, formation of sulfhemoglobin. The

effects produced are dependent on 1, length of time of exposure; 2, area of exposure; 3, manner of skin preparation, and 4, amount applied.

Grossly, the exposed skin exhibits varying degrees of erythema and inflammation, with "slate grey" duskiness. Histologically the significant lesion is a severe necrotizing dermatitis.

Penetration of ammonium hydrogen sulfide is relatively rapid; hydrogen sulfide alone slow. These experiments do not decide whether ammonia facilitates passage of hydrogen sulfide by virtue of its irritant properties to skin or to the formation of molecular ammonium hydrogen sulfide.

Influence of anesthetics on balance between chemoreceptor and central control of respiration. PAUL DUMKE and ROBERT DRIPPS. *Laby. of Pharmacology, Univ. of Pennsylvania.* In decerebrated dogs and cats reactivity of respiratory center was estimated by effect of inhalation of CO_2 (10 per cent in O_2), that of chemoreceptors by smallest dose of NaCN that elicited distinct hyperpnea when injected intravascularly. Barbital, pentobarbital, pentothal, morphine, chloralose, ether, or cyclopropane was then given and tests were repeated. All drugs were found to decrease reactivity of center to CO_2 in approximate proportion to depth of narcosis. Sensitivity of chemoreceptors to cyanide was reduced by ether and cyclopropane, unaltered or increased by the other drugs. In 7 intact trained dogs chloralose was found to act as it did in decerebrates. Oxygen inhalation diminished reactivity of chemoreceptors to cyanide but not to lobeline, which confirms Euler's and Gesell's belief that the chemoreceptors are specialized with respect to different stimuli. By means of faradic stimulation through electrodes placed on nerve fibers between carotid sinus and carotid body in dogs effectiveness of impulses from pressoreceptors to centers controlling circulation was found to be unaltered or augmented by chloralose, diminished by ether; such stimulation also caused apnea in chloralosed dogs but this effect was entirely abolished by ether or by decerebration although typical effects were still exerted on blood pressure and pulse rate. This indicates that respiratory effects from pressoreceptors involve higher parts of neuraxis while circulatory effects do not, and that ether blocks spread of impulses more than other drugs. The possible significance of these findings will be pointed out.

Serial determinations of cardiac output (ballistocardiograms) and electrocardiogram in normal man after the administration of digitalis glycosides. LUDWIG W. EICHNA AND HARRY TAUBE. *New York Univ. College of Medicine.* In normal man a fall in cardiac output has been observed following the oral administration of digitalis whole leaf. The present study in normal subjects had two purposes: 1, to determine whether the purified glycosides produce a similar affect, and 2, to compare the various glycosides with each other in this regard. Ballistocardiographic determination of cardiac output and intravenous administration of the glycosides in single therapeutic doses permitted frequent serial measurements throughout the entire period of action of the drug. Simultaneous electrocardiograms and ballistocardiograms were taken three to four times during the first hour after injection, ten to twelve times in the first eight to ten hours, and thereafter daily or every other day up to two weeks. Seven subjects received digoxin (1.5 mgm. to 2.5 mgm.); seven, lanatoside C (1.5 mgm. to 2.5 mgm.); and three, digitaline Nativelle (digitoxin) (1.4 mgm. to 1.8 mgm.). Four subjects received more than one glycoside; the interval between injections exceeded two weeks.

In most subjects depression of T waves in one or more leads followed the administration of each glycoside. After digoxin and lanatoside C these changes began within 15 to 25 minutes, reached a maximum in one to six hours, evidenced regression in 24 to 30 hours (occasionally in 8 to 10 hrs.), and returned to normal in 2 to 3 days or longer. Following digitoxin the changes appeared more slowly and persisted longer.

The changes in cardiac output were small (10 to 20 per cent) and not necessarily related to the electrocardiographic deviations. Cardiac rate rather than stroke output determined the minute output. Usually stroke output increased, but minute output fell slightly as the heart rate decreased. Occasionally the heart rate did not fall, the minute output then remained unaltered.

The inhibition of cholinesterase by breakdown products of physostigmine. SYDNEY ELLIS. *Dept. of Pharmacology, Harvard Medical School, Boston, Mass.* Recent studies from this laboratory showed that when physostigmine was allowed to decompose in buffer solution, in horse or dog serum *in vitro*, or in dogs *in vivo*, the inhibitory action of physostigmine on cholinesterase was lost entirely after an interval dependent on the medium. Subsequently, however, an inhibitory action reappeared. We made a detailed study of the breakdown products of physostigmine to find compounds which would inhibit cholinesterase.

The course of physostigmine breakdown is as follows: physostigmine loses its methylcarbamyl group by hydrolysis and forms eseroline, which in turn absorbs two atoms of oxygen to form rubreserine, a red crystalline compound with an orthoquinone structure; rubreserine or any of its precursors, on long standing or with drastic conditions, is converted to eserine blue (blue in basic and strong red fluorescence in acid solution).

The end product of the breakdown is eserine brown (Salway. J. Chem. Soc. **101**: 978, 1912), a complex mixture.

We have studied the cholinesterase inhibition of the various breakdown products. Eseroline, an inhibitor in relatively high concentrations (Schweitzer, Stedman and Wright. J. Physiol. **96**: 302, 1939), has no inhibitory activity in the range studied. Rubreserine and eserine blue, however, show a definite cholinesterase inhibition in concentrations that would account for the unusual return of cholinesterase inhibition in decomposing physostigmine solutions.

The possibility of physostigmine contamination causing the inhibitory action attributed to these substances was eliminated by dilution experiments. Conversion of an inactive eseroline solution to an active solution gave further evidence that rubreserine and eserine blue are active inhibitors of cholinesterase.

Effects on coronary circulation of isomers of neosynephrine and of amphetamine. R. A. ESCOBAR, H. F. WILKINS and C. H. THIENES. *Univ. of Southern California.* Coronary blood flow in unanesthetized dogs was measured by means of a modification of the direct current thermostromuhr of Baldes and Herrick. Four dogs, weighing 15 to 20 kilograms each were used. Neosynephrine (levo-meta-synephrine) and its optical isomer, dextro-meta-synephrine, and levo- and dextro-amphetamine were administered intravenously and intraperitoneally in doses of 0.3 to 2 mg. per kilo. Except in a few trials, dextro-meta-synephrine increased coronary blood flow whereas neosynephrine decreased coronary flow. The opposite relations were observed with the amphetamines in that the levo-form increased coronary blood flow and the dextro-form decreased the flow. [*Aided by Works Projects Administration project no. C. S.*-12295 *and by a grant from Smith, Kline and French Laboratories.*]

A uterine principle from viburnum prunifolium. WILLIAM E. EVANS, JR. *Dept. of Pharmacology, School of Medicine, Univ. of Maryland.* A substance was isolated from Viburnum Prunifolium which reversibly depressed or abolished contractions of human and rat uterine muscle. The influence of atropine and of cocaine on the response of uterine tissue to the drug was investigated.

The effect of a number of new phenyl-piperidine derivatives on smooth muscle. EDWIN J. FELLOWS and RAYMOND W. CUNNINGHAM. *Temple Univ. School of Medicine.* A number of new compounds (hydrochlorides of α and β forms of 4-methyl-, 4-dimethyl-, 4-methylol-, α and β forms of 4-phenyl-, 4-carboxylic acid, 4-carbomethoxy-, and 4-carbethoxy-modifications of 3-phenyl-piperidine) were examined for antispasmodic activity. The present derivatives were found to relax normal smooth muscle and also that made spastic with histamine, pituitrin, furfuryl trimethyl ammonium iodide or barium chloride. Comparisons of effectiveness and toxicity of the phenyl-piperidine compounds were made with papaverine hydrochloride.

Comparative toxicity of kerosenes. J. K. FINNEGAN, E. L. MCCAWLEY and L. A. STRAIT. *Univ. of California Medical School.* During toxicity studies on various fly-spray preparations it was noted that the oral toxicities of these meterials may be correlated more consistently with the type of kerosene vehicle used than with the various and relatively non-toxic insecticides incorporated in them. Two types of kerosenes were studied: "Mid-Continent" and "West Coast." Inhalation toxicity was investigated on mice in a specially designed spray chamber. Absorption of the spray was not rapid enough to cause significant symptoms on 60 minutes exposure daily for several days. No untoward symptoms occur on single oral administration of either type of kerosene in mice in amounts taxing their stomachs' capacities (50 cc. per kgm.). However, at a daily oral dose of 5 cc. per kgm., 6/10 of the test animals were dead after the sixth administration of Mid-Continent kerosene, whereas none of those receiving West Coast kerosene had died. All mice given Mid-Continent kerosene daily at this dose were dead within two weeks, with progressive and marked weight loss. On the other hand, 6/10 of those given West Coast kerosene in the same manner were still alive after 4 weeks, with insignificant weight changes. Spectrographic examination of the two types of kerosenes reveals a chemical difference other than that expected from a homologous series of aliphatic hydrocarbons. We have not yet succeeded in isolating the toxic factor by fractional distillation.

Reaction of fallopian tubes and ovaries of rabbits to repeated administration of Pitressin NNR. PAUL G. FUERSTNER. *Univ. of California Medical School.* Nedzel (Arch. Path. **26**: 988, 1938) showed that repeated local tissue anoxia from vascular constriction produces prompt and characteristic histological changes. Experiments were undertaken to determine whether or not female genital tissues might show such effects from such causes. In female rabbits after single or repeated intravenous injections of 0.5 to 2.0 cc. of Pitressin NNR for 5 to 10 times at 1 to 3 day intervals in 25 animals in comparison with 10 controls, there was uniformly found to be congestion, hyperemia, and round cell infiltration in the fallopian tubes, with a similar inflammatory reaction in the ovaries and a great increase in Call-Exner bodies.

A method for the bio-assay of digitalis in humans. HARRY GOLD, HAROLD OTTO and NATHANIEL T.

KWIT. *Dept. of Pharmacology, Cornell Univ. Medical College and the Cardiac Clinics of the Hospital for Joint Diseases and Beth Israel Hospital, New York City.* In previous investigations evidence was presented for the view that animal methods for the assay of digitalis bodies may be misleading when the results are applied to humans. It was shown that the frog method has serious defects in this respect, since a specimen may be twice as active as another when compared in the frog, while of the same activity when compared in humans. In these studies the results of the cat method proved to be a more reliable index of the relative potency of digitalis preparations in man. Defects of the cat method were also pointed out. In the past two years we have explored the possibilities of using human subjects for the assay of digitalis. Degrees of change in the T-wave of the electrocardiogram in patients with regular sinus rhythm indicate grades of digitalis action. In suitable individuals the T-wave reliably reveals 22 per cent differences in dosage. The present technique is to calibrate the patient with three doses of the standard Reference Powder, differing from each other by 22 per cent. The dose of the unknown is then selected which produces an effect falling within the calibrated range. The ratio of the dose of the unknown to the dose of the standard represents the relative potency of the two preparations. The average of these ratios for a series of patients establishes the potency of the unknown in relation to the standard. Up to the present time, 97 subjects have been used for calibration. About 20 per cent of these have proved sufficiently constant in their response to digitalis for a dependable estimate of the potency of the unknown specimen. Eight preparations of digitalis have been satisfactorily assayed by this method.

Tolerance and cross tolerance to barbiturates. MELVIN W. GREEN and THEODORE KOPPANYI. *Dept. of Pharmacoloty and Materia Medica, Georgetown Univ., School of Medicine, Washington, D. C.* The question of tolerance to barbiturates is still moot. Dogs were given thrice weekly and later once daily 30 mgm /kgm. of evipal-sodium; another group of dogs 20 mgm./kgm. pentothal sodium daily and a third group 30 mgm./kgm. of nostal sodium daily, intravenously. After about the tenth injection the average sleeping time of the evipal-treated dogs was reduced from 57 to 9 minutes. The sleeping time of the pentothal-treated dogs fell from 95 to 30 minutes, while the nostal-treated dogs after the fourth or fifth injection did not go to sleep at all. Evipal-tolerant dogs when narcotized with pentothal showed an average sleeping time of 23 instead of 95 minutes, while nostal-tolerant dogs slept for 47 minutes following pentothal and 5 minutes following evipal. The nostal-tolerant dogs also showed tolerance to the long-acting barbiturate, barbital-sodium. While controls slept 35 hours following 225 mgm./kgm. barbital sodium, the sleeping time observed in the nostal-tolerant dogs following the same dose of barbital was only 10.5 hours on the average. A fatal dose of 500 mgm./kgm. of sodium barbital caused death in the controls in an average time of 152 minutes while the same dose of barbital was survived by the nostal-tolerant dogs for 320 minutes. The tolerance to barbiturates is short-lived, for it greatly diminishes or entirely disappears within 96 hours. While tolerance is established to the central nervous actions of barbiturates no tolerance could be observed to peripheral barbiturate actions such as effects on vasodepression following intravenous injection of evipal, vagal paralysis, pressor responses to epinephrine and carotid occlusion.

The papain activating power of blood in health and disease. CHARLES GURCHOT and MICHAEL JOSEPH. *Univ. of California Medical School.* Purr and Russell reported (Ztschr. Physiol. Chem. **228:** 198, 1934) that blood from cancer patients and tumor-inoculated rats activates papain significantly less than blood from healthy controls. We studied papain activation by photoelectric measurement of the amount of carmine liberated by the enzyme from specially prepared dyed gelatin buttons. We found that papain is activated by blood from patients suffering from diabetes, syphilis, arteriosclerosis, hypo- and hyper thyroidism, liver disease and kidney disease to the same extent as by blood from healthy people. Activation by blood from women in late pregnancy or from persons with benign tumors is slightly less. Activation by blood from women in early pregnancy, or from persons with active tuberculosis or untreated malignancy is less than half that caused by blood from normal individuals.

Urinary excretion of nicotine by smokers. H. B. HAAG and P. S. LARSON. *Dept. of Pharmacology, Medical College of Virginia, Richmond.* Urinary excretion of nicotine was determined chemically (and confirmed in several instances biologically) in four smokers. The subjects smoked 40 cigarettes per day and eliminated from 2 to 8 mgm. of nicotine in the urine. This is the equivalent of about 2 to 8 per cent of the nicotine retained on inhalation of the tobacco smoke. Urine from two non-smokers showed the presence of a trace of material giving a positive nicotine test (0.01 to 0.08 mgm. per 24 hour sample).

In one subject studied especially for the purpose, acidification of the urine by the oral administration of ammonium chloride increased the urinary yield of nicotine (from an average of 6.4 mg. per 24 hours during the uncontrolled period to an average of 15 mg.); alkalinization of the

urine decreased the urinary yield (to an average of 2.7 mgm.). The extent of this increased resorption is too slight however to support the suggestion that poisoning might occur in a normal smoker as a result of massive resorption of nicotine by the urinary mucous membrane from an alkaline urine.

Under like experimental conditions, a non-inhaling smoker excreted in the urine only 10 per cent as much nicotine as an inhaler. This is in keeping with the observation that a non-inhaler retains only about 10 per cent as much nicotine as an inhaler, based on assay of the expelled smoke.

It is concluded from the present experiments that some more effective mechanism than urinary excretion is normally active in the disposal of nicotine by man.

Effects of benzedrine on oxygen consumption after morphine in men and rats. CARROLL A. HANDLEY and BENEDICT E. ABREU. *Univ. of California Medical School, Univ. of South Dakota School of Medicine and Univ. of Georgia School of Medicine.* Previous studies (Anesthesia and Analgesia **20**: 212, 1941) indicate that benzedrine is an effective medullary and cortical stimulant in rabbits depressed by morphine. Morphine sulfate 0.3 mgm./kgm. orally produced in man a slight decrease in oxygen consumption which was not antagonized by benzedrine sulfate 0.3 mgm./kgm. orally. However, cortical depressant effects from this dosage of morphine, as measured by the tactile response to pain using Chéron's instrument, were abolished within 30 minutes by this dosage of benzedrine. No significant cardiac or pressor effects were observed when morphine was followed by this dosage of benzedrine.

The oxygen consumption of white rats was determined by the Peoples Method. Benzedrine sulfate 10 mgm./kgm. intraperitoneally significantly stimulated the oxygen consumption of animals which had received morphine sulfate 20 mgm./kgm. intraperitoneally.

The pyretic action on rats of small doses of morphine. JULIAN B. HERRMANN. *Yale Univ. School of Medicine.* The subcutaneous administration of small doses of morphine sulphate, 1 to 30 milligrams per kilo, consistently produces in rats a moderate pyrexia. Larger doses, 40 to 90 mgm. per kilo, produce only hypothermia. The pyrexia is associated with catalepsy and exophthalmos. With an average temperature rise of 2.6°C. there is an increase in metabolic rate of 10 per cent which is more than accounted for as a result of the temperature rise (Vant Hof's Law). However, this 10 per cent metabolic increase accounts for only 20 per cent of the calories necessary to produce the pyrexia; the balance is therefore derived from heat conservation.

The morphine pyrexia and its concomitant exophthalmos may be prevented by preliminary bilateral adrenalectomy or by ergotization. Also, adrenalectomized rats, which exhibit reduction in metabolic rate, fail to show metabolic increase after morphine. Adrenodemedullation, alone or in conjunction with hypophysectomy, on the other hand, does not prevent the appearance of the morphine fever, catalepsy and exophthalmos.

The administration of adrenal cortical extract or 0.9 per cent NaCl to adrenalectomized animals restores their ability to develop a morphine pyrexia; contrariwise, epinephrine apparently does not.

Bilateral adrenalectomy in rats enhances the toxicity of morphine whereas adreno-demedullation or hypophysectomy fails to influence it.

The addiction liability of demerol (1-methyl-4-phenyl-piperidine-4-carbonic acid ethyl ester). C. K. HIMMELSBACH. *U. S. P. H. S. Hospital, Lexington, Ky.* Demerol was substituted for morphine and administered to 13 addicts for 10 days. In each instance physical dependence on morphine was partially satisfied by Demerol. On withdrawing Demerol, the abstinence syndrome became intensified for two days, then waned.

Demerol was given to a group of "recovered" or post-addicts in progressively increasing amounts for 10 to 11 weeks. Signs of withdrawal appeared on withholding the drug after one and two months of administration. The abstinence syndrome which appeared on withdrawal was in every way typical of, but less severe than the morphine abstinence syndrome.

The maximum daily dose was 3.5 grams. The duration of its physical dependence action was 4 to 5 hours (the time required for the abstinence syndrome to reach 50 per cent of its maximum intensity).

Muscular tremors and twitches appeared in the second week and continued throughout. In the 10th week two of the subjects had mild epileptiform (petit mal) seizures.

Metabolism of some derivatives of dilantin. C. H. HINE, J. H. HOLMAN and F. L. KOZELKA. *Dept. of Pharmacology and Toxicology, Univ. of Wisconsin, Madison.* We have reported previously that dilantin is metabolized in man and dog to the corresponding hydantoic and amino acids. To determine the effect of various substituent groups on the stability of the hydantoin nucleus and on its anticonvulsant activity, the following derivatives were prepared: 1-acetyl, 2-thio, 3-methyl, and the 5,5 dianisyl and 5-ethyl 5-phenyl hydantoins.

These compounds were administered parenterally in divided doses to dogs. The urine was collected for a period of 48 hours after the last dose

and examined for the presence of the unchanged compound and its degradation products. The techniques employed for the recovery of each compound were tested previously on prepared urine specimens and found adequate for the purpose. With the exception of the 5-ethyl 5-phenyl derivative, the percentage excretion of the unchanged compound, the hydantoic acid, and the amino acid corresponded approximately to that found after the administration of dilantin. Approximately 15 per cent of the 5-ethyl 5-phenyl derivative appeared in the urine unchanged. Apparently the presence of the methyl group fails to prevent the cleavage of the nucleus between the *3* and *4* positions. The further degradation of the diphenylhydantoic acid and amino acid appears probable since they are not entirely excreted unchanged. Diphenyl amino methane and benzophenone, two possible degradation products of the latter compounds, likewise cannot be demonstrated in the urine and feces.

A suggested pharmacological basis for the widely varying toxicity of arsenicals. RALPH B. HOGAN and HARRY EAGLE. *U. S. Public Health Service, Washington, D. C., and the Syphilis Division of the Dept. of Medicine, Johns Hopkins Medical School, Baltimore, Md.* Organic arsenicals added to whole blood *in vitro* are absorbed by the red blood cells at varying rates and to varying degrees. In general, the affinity of the arsenical for the cells parallels its toxicity. It is therefore suggested that the enormously varying toxicity of organic arsenicals may rest simply on the varying degree to which they are taken up and held by the body tissues.

Statistical studies upon the effects of anesthetics in the intravenous cat method of standardizing digitalis. HARALD G. O. HOLCK, E. L. SMITH, R. H. SHULER, R. VAN NORMAN, D. R. MATHIESON and J. DAHLSTET. *Dept. of Physiology and Pharmacology, College of Pharmacy, Univ. of Nebraska, Lincoln.* Extension of our statistical studies upon the digitalis bioassay by the intravenous cat method under standardized conditions show that: 1. The ratio of the higher value obtained with pentobarbital (nembutal) (courtesy of the Abbott Laboratories) anesthesia is about the same when the average dying time is 47 and 92 minutes, respectively; 2. The values resulting with artificial respiration and recording of blood-pressure do not differ significantly from those secured with the simpler auscultatory method, using pentobarbital anesthesia; 3. The values with diallylbarbiturate (donated by Ciba Pharmaceutical Products) anesthesia do not differ significantly from those obtained with pentobarbital anesthesia, but with dial distinct tremors occur in many cats; 4. The values with urethane anesthesia fall about midway between those seen with pentobarbital and with ether anesthesia, respectively.

The barbiturates were administered intramuscularly as 5 per cent solutions of their respective sodium salts; the urethane was given intraperitoneally as a 33 per cent solution in water. [*Aided by a grant from the Committee on Therapeutic Research, Council of Pharmacy and Chemistry, American Medical Association, and by a grant from the Nebraska Foundation.*]

The eserine-like action of certain new urethane methiodides. NORMAN W. KARR. *Univ. of California Medical School.* m-Isopropyl-p-dimethylamino-phenol-dimethyl-urethane methiodide, p-dimethylamino-thymol-dimethyl-urethane methiodide, and p-dimethylamino-carvacrol-dimethyl-urethane methiodide were furnished by the Merck Research Laboratory for comparison with eserine. *In vitro* hydrolysis of acetyl-choline by horse serum is completely inhibited by 0.001 per cent solution of physostigmine, prostigmine, or any of the three new compounds. The new synthetics in equivalent molecular doses produce greater depression of respiration and blood pressure than physostigmine salicylate in doses of 0.01 to 0.1 mgm./kgm. The phenol derivative seems to be most active. In concentrations of 0.001 per cent in Adler-Tyrode solution, physostigmine and each of the three synthetics produce marked but transient increase in tone of isolated gut from rats, guinea pigs, and rabbits. Skeletal muscle paralyzed in the intact animal by beta-erythroidine may be restored by physostigmine or any of the new synthetics in doses from 0.01 mgm./kgm. to 0.15 mgm./kgm. The thymol derivative and physostigmine act slowly even in large doses. The carvacrol compound is faster, while prostigmine and the phenol compound show a very rapid restoration and improvement of activity of the *tibialis anticus* beyond normal performance even though the stimulus remains constant in intensity.

Histamine release in the allergic skin reaction. GERHARD KATZ. *Dept. of Pharmacology, Tulane Univ. of Louisiana School of Medicine.* It was shown in previous investigations that blood cells from allergic patients release histamine in vitro upon contact with the specific allergen. (G. Katz and S. Cohen; J. A. M. A. 117: 21, 1941.) The following experiments were designed to extend these studies to the allergic skin reaction in situ. Cantharides plasters were applied to chest or arm of ragweed allergic individuals. After 15 to 24 hours, the dead skin over the newly formed blister was removed; the denuded area was covered with an inverted funnel which was held in position by a holder which had been fastened to the skin with collodion. Locke's solution was introduced into the funnel and left for constant periods. It was then replaced by the same volume

of fresh Locke's. The samples were assayed for histamine on the isolated atropinized guinea pig's ileum either directly or after extraction (according to Barsoum, Gaddum-Code). After the control period, ragweed extract was injected into the denuded area. In the cases in which a distinct local reaction occurred (which, with the ragweed dosage employed, was usually followed by some systemic reactions), the histamine content of the fluid rose within the first ten minutes. It reached its peak within 30 minutes and cropped to the original level within 60 minutes after introduction of the allergen. Parallel with this histamine release there occurred an increase in protein content of the supernatant fluid as determined by trichloroacetic acid precipitation. An increased rate of fluid excretion and the appearance of a substance like the "slow reacting substance" of Feldberg and Kellaway were also observed in the allergic skin reaction. [*Aided by a grant from the John and Mary R. Markle Foundation.*]

The comparative metabolic effects of testosterone propionate and estradiol benzoate in man. KATHRYN KNOWLTON, IRENE SANDIFORD and ALLAN T. KENYON. *Dept. of Medicine, Univ. of Chicago, Ill.* Previous work of our own and others has shown that 25 mgm. of testosterone propionate given daily intramuscularly to the sexually underdeveloped man or woman will induce reduction in urinary excretion of nitrogen, inorganic phosphorus, inorganic sulfate, sodium, potassium and creatine and will increase basal heat production. These effects could not be explained by growth of the genitalia and were accordingly termed "somatotropic" in an intentionally loose sense. Five milligrams of testosterone propionate per day, an amount well below most estimates of replacement requirements, will produce distinct influences of this character in sexually underdeveloped men. Five milligrams of estradiol benzoate daily in sexually underdeveloped men or women will reduce urinary nitrogen and inorganic phosphorus excretion about as much as will a comparable amount of testosterone propionate. As with testosterone there is no increase in the concentration of these substances in the blood. Urinary sodium is reduced; urinary potassium and inorganic sulfate are not conclusively affected. When creatinuria is sustained by creatine ingestion estradiol benzoate does not decrease the creatinuria as does testosterone. No distinct modification of heat production was induced by the estrogen. Since the amounts of estradiol given are excessive from the physiological standpoint it is not clear that these properties of inducing nitrogen and phosphorus retention are normally utilized by the organism as are the similar properties of testosterone. [*This work was supported in part by a grant from the Committee on Research in Endocrinology of the National Research Council.*]

Studies on peripheral blood flow throughout the cardiac cycle the electromagnetic method. ALEXANDER KOLIN and DAVID W. FASSETT. The electromagnetic method of measuring blood flow (A. Kolin Proc. Soc. Exper. Biol. and Med. **46**: 235, 1941; Rev. Sci. Instr. **12**: 555, 1941) has been used to study the peripheral blood flow in dogs and cats throughout the cardiac cycle under normal conditions and under the action of various pharmacological stimuli. In this report effects of various vasoconstrictors and dilators on blood flow through the femoral, external and internal carotid arteries will be presented. Among the numerous drugs tested epinephrine and acetylcholine were studied in more detail.

Intravenous administration of drugs causing an abrupt drop of blood pressure caused a reduction in diastolic flow and, in greater doses, a diastolic regurgitation. This effect depends on the rate of fall of pressure rather than pressure value.

In order to study the effect of drugs on the peripheral resistance without the complications of simultaneous pressure changes, drugs were also injected intra-arterially. Doses too small to affect systemic blood pressure could be used. Thus it was found, for instance, that 0.0001 γ of acetylcholine will about double the femoral flow in a dog. It will take, however, 50 γ to produce a comparable flow change in the internal carotid where the normal flow is about one-fifth as large. A similar difference in the sensitivity of these two regions was also displayed toward epinephrine.

Contributions to the pharmacology of sulfides. THEODORE KOPPANYI and C. R. LINEGAR. *Dept. of Pharmacology and Materia Medica, Georgetown Univ., School of Medicine, Washington, D. C.* Alkaline sulfides (Na_2S) upon intravenous injection in doses from 0.5 to 10 mgm./kgm. produce sharp fleeting rise in blood pressure (from 10 to 150 mm.Hg) and marked stimulation of the rate and depth of respiration. In eserinized or prostigminized mammals the pressor effect is preceded or replaced by a fall in blood pressure depending upon a marked slowing of the heart. The vagus stimulating effect of Na_2S is central, for it is prevented or abolished by double vagotomy. The pressor effect is peripheral, for it is not abolished by nicotine (10 mgm./kgm.) or sparteine (20 mgm./kgm.) and is not due to epinephrine mobilization from the adrenals since it persists following clamping of the adrenal veins. The respiratory stimulating effect of Na_2S is not prevented by sectioning both vagi in the neck nor by extirpation of the carotid bodies, but is abolished by sectioning the vagi above the ganglia nodosa. Doses of nicotine large enough to abolish the respiratory stimulating effects of acetylcholine or

nicotine itself do not interfere with respiratory stimulation by sulfides.

Commercial preparations of colloidal sulfur possess identical pharmacological actions owing to their alkaline sulfide content. Shaking these preparations with lactic acid diminishes or abolishes their hemodynamic and respiratory effects. Allyl sulfide in any dosage produces vasodepressor effects only.

Anticonvulsant properties of the metabolic products and some derivatives of dilantin. F. L. KOZELKA, C. H. HINE and M. F. GREIBER. *Depts. of Pharmacology and Toxicology and Neuropsychiatry, Univ. of Wisconsin, Madison.* The anticonvulsant properties of alpha amino diphenylacetic acid, diphenylhydantoic acid, diphenylamino methane, 5,5 dianisyl hydantoin, and the 1-acetyl, 2-thio, and 3-methyl derivatives of 5,5 diphenyl-hydantoin were determined. The anticonvulsant effect of these drugs was measured on cats by the increase which was produced in the threshold (time the stimulus must be applied to produce a convulsion) to an electrical stimulus having a constant amperage and resistance. A shock therapy apparatus was employed for this purpose. The cats received a single daily dose orally for four days and were tested three hours after the first and fourth doses. In order to maintain sensitivity and uniformity, only cats with a normal threshold of 0.05 to 0.1 second were employed.

All these drugs, with the exception of the dianisyl derivative, were effective in increasing the threshold. The increase in the threshold was two- to tenfold. These results indicate, when correlated with metabolism studies, that compounds capable of yielding metabolic products similar to those of dilantin are effective as anticonvulsants. Clinical studies on epileptic patients are in progress.

The pharmacology and anesthetic properties of cyclopropyl vinyl ether. JOHN C. KRANTZ, JR. and SYLVAN E. FORMAN. *Dept. of Pharmacology, School of Medicine, Univ. of Maryland.* Cyclopropyl vinyl ehter (cyprethylene ether) is a compound containing structures essential to the molecules of the three anesthetics cyclopropane, ethyl ether and ethylene. Thus cyprethylene ether contains the cyclopropyl ring, the carbon-oxygen-carbon linkage of ethyl ether and the double bond of ethylene.

Cyprethylene ether is an anesthetic in man and many species of animals. Its potency is approximately the same as divinyl oxide. The anesthetic index of cyprethylene ether is very high, approximately twice that of diethyl ether.

The effect of veratrum alkaloids on circulatory reflexes. OTTO KRAYER. *Dept. of Pharmacology, Harvard Medical School, Boston, Mass.*, The circulatory reflexes caused by veratrum alkaloids were studied by using the innervated heart-lung preparation as described by Krayer and Verney (Arch. f. exper. Path. u. Pharmakol. **180:** 75, 1935). This method allowed the investigation of the reflex reaction due to the influence of the alkaloids upon receptors in the heart-lung circulation separate from those reactions originating in other areas of the autonomic nervous system of the neck and head region.

The pure alkaloid veratridine, as well as veratrine, caused a reflex decrease in heart rate when applied to the heart-lung circulation, which was in nervous connection with the central nervous system only by way of the *nervi vagi*. One-hundredth milligram of veratridine (veratric acid ester of cevine) produced a marked effect, while cevine, in a dose 100 times larger, had no significant action.

The action of the xanthines on the force of contraction of isolated mammalian heart muscle. STEPHEN KROP. *Dept. of Pharmacology, Cornell Univ. Medical College, New York City.* The action of caffeine, theophylline and theobromine on the force of contraction of the isolated papillary muscle of the cat's right ventricle was studied. The drugs (in the form of free base and of salts, i.e., with sodium benzoate, sodium salicylate, ethylenediamine and sodium acetate) were introduced directly into the oxygenated Locke's solution after a control time interval.

No significant increase in "systolic" force resulted from caffeine except in concentrations of 1:1,000 or higher. Under such conditions there was a 50 to 250 per cent increase, depending on the concentration, in the "systolic" tension, which was rapid in onset (2 to 15 min.) and brief in duration (5 to 30 min.). Theobromine produced similar effects in lower concentrations (1:2,000 to 1:1,000). Theophylline exhibited similar properties and was the most effective (1:5,000). The effects of the drugs were apparently unrelated to changes in "tone."

Therefore, in concentrations comparable to those obtaining in the blood stream of man after intravenous injection of therapeutic doses (about 1:10,000 at the time of injection) the xanthines produce no significant changes in the force of contraction of heart muscle of the cat. With respect to their action in increasing the force of contraction of cardiac muscle, the xanthines differ from the digitalis glycosides in requiring relatively high concentrations and in acting briefly.

The arsenic analogue of choline as a component of lecithin in rats fed arsenocholine chloride. R. L. LANDAU and A. D. WELCH. *Dept. of Pharmacology, Washington Univ. School of Medicine, St. Louis, Mo.* Acetylarsenocholine can substitute qualitatively for acetylcholine and arsenocholine exerts lipotropic, kidney anti-hemorrhagic

and anti-perotic activity nearly equivalent to choline, although it is unable to transfer methyl groups to homocysteine. Arsenic was demonstrated spectrographically in phospholipids obtained from animals fed arsenocholine chloride, which suggested that the arsenic analogue is utilized in the bio-synthesis of phospholipids. The decomposition which arsenocholine undergoes in the body, probably to trimethylarsine, indicated the need for further evidence with regard to the nature of the arsenic compound in phospholipids. Lecithin from rats fed arsenocholine chloride was hydrolyzed and treated with ammonium reineckate; the precipitate, after extraction with absolute ethanol, was decomposed and a crystalline gold salt obtained which analyzed correctly for a mixture of the aurichlorides of choline and arsenocholine in a ratio of 18:1. The decomposed gold salt yielded a periodide precipitate under conditions highly specific for choline; this precipitate contained arsenic. The evidence appears adequate to justify the original conclusion that arsenocholine, and therefore exogenous choline, enters directly into lecithin synthesis. Stetten's work with choline containing N^{15} favors a similar conclusion. The occurrence of arsenocholine in lecithin and its failure to serve as a donor of methyl groups suggest that reactions involving the intact molecule are responsible for the lipotropic and anti-hemorrhagic actions of arsenocholine. The same conclusion may logically be applied to choline. Transmethylation reactions appear to be involved in choline synthesis but they apparently are not concerned with the lipotropic and kidney anti-hemorrhagic actions of the compound.

The estimation of the safety margin of inhalation agents. C. D. LEAKE. *Univ. of California Medical School.* Experimentation with various methods of estimating the safety factor of inhalation agents, such as war gases, volatile anesthetics or industrial solvents, indicates that the most practically significant and accurate is by use of time-concentration data. Definite molecular concentration mixtures may be maintained by flowmeters calibrated for each gas in air or oxygen. Test animals may be placed in suitable glass chambers through which the desired concentration continues to flow without accumulation of water-vapor, heat, or carbon dioxide. Appropriate symptoms may be taken as objective criteria for the onset of mucous membrane irritation, central nervous system depression, delirium, anesthesia, coma, and respiratory failure, in the same test organism exposed to a constant concentration. The onset of these progressive conditions may readily be timed. The use of sufficient animals at representative concentrations may furnish data adequate for statistical treatment for the plotting of time-concentration curves for each condition for which onset times are recorded. The distance between these curves may be taken as reliable indices for the particular sort of "safety margin" one may prefer. Such time-concentration curves furnish a ready means of comparing the relative safety factors of different compounds.

The anticonvulsant action of natural and synthetic curare-like agents. A. J. LEHMAN and H. F. CHASE. *Wayne Univ. College of Medicine, Detroit, Mich.* The anticonvulsant action of quinine ethochloride dihydrate (QEC), quinine methochloride (QMC), beta-erythroidine hydrochloride (BE), and dihydro-beta-erythroidine (DBE) was determined in dogs using metrazol, 25 mgm/kgm., as the convulsant. Dogs could not be as satisfactorily protected with BE, 5.0 mgm/kgm., DBE, 1 mgm/kgm., and QMC, 4.5 mgm/kgm., as with QEC, 8.5 mgm/kgm. Toxic effects of the latter were not as manifest as with the other three compounds. Higher doses of BE, DBE, and QMC gave better protection than the smaller doses listed, but the resulting respiratory embarrassment necessitated artificial respiration to save the animal. A strong parasympathetic stimulation, possibly nicotine-like, was noted for BE and DBE as an undesirable side-action. QEC, in the dose given, limited metrazol convulsions to a few mild tremors and resuscitative measures were not necessary. In our hands prostigmine affords little or no antagonism to the curare-like actions of any of these agents.

The curare-like action of certain new synthetic agents. A. J. LEHMAN and H. F. CHASE. *Wayne Univ. College of Medicine, Detroit, Mich.* A series of about forty synthetic agents was tested for their curare-like effects. Among these compounds were new quarternary ammonium bases, derivatives of quinine, quinoline, brucine, nicotine, pyridine, morpholinium, and picoline. Two naturally occurring alkaloids, beta-erythroidine hydrochloride and dihydro-beta-erythroidine hydrobromide were also studied. The degree of muscular paralysis was observed in amphibia, fowls and mammals. Central and peripheral respiratory effects and cardiovascular changes were determined in dogs by the method of Thomas and Franke. The most promising synthetic agent is quinine ethochloride dihydrate; dihydro-beta-erythroidine hydrobromide is the most potent natural compound.

A practical technique and design for the assay digitalis on the embryonic chick heart. ROBERT A. LEHMAN and GEORGE H. PAFF. *New York Univ., College of Medicine.* In a previous report it was shown that the potency ratios for lanatoside C, digoxin, and digitaline Nativelle obtained on the embryonic chick heart agree much better with the human oral dose than do those obtained on the

cat or the frog. This work has been extended to the assay of digitalis tincture and powdered leaf for which a practical procedure is described. The sources of variation affecting the assay are evaluated and the method is shown to compare favorably with other commonly used methods with respect to precision, time consumption, cost, and availability of the biological material. An experimental design is suggested whereby use may be made of factorial coefficients as a short cut in the computation of the ratio of standard to unknown and its standard error. Appropriate tests of significance are indicated. Comparisons are made between the potency of various preparations for the embryonic heart, the cat, the frog, and man. The chick heart results have been found to parallel closely the human in so far as therapeutic data were available. They also agree with the cat in many but not all cases. Preliminary data suggest that tinctures which have aged with respect to the frog, retain their potency for the chick heart.

Tolerance to repeated doses of acetins in rats and mice. R. C. Li. *Peiping Union Medical College.* In previous studies (Proc. Soc. Exper. Biol. and Med. **46:** 26, 1941; Am. J. Physiol. **72:** 26, 1941) the acute toxicity of the 3 glyceryl acetates was determined in the rat, mouse, dog and rabbit. The acute toxic range differed somewhat with different species, but it was believed that an increase in toxicity occurred with increase in acetylation. The effects of repeated subcutaneous injections of the 3 acetins in the rat and mouse are reported. Doses equivalent to $\frac{1}{10}$ of LD_{50} were given subcutaneously 3 times weekly until the amounts were equivalent to LD_{100}. One of 20 rats died after monacetin, 3 of 20 after diacetin and none of 20 after triacetin. Animals died of hemorrhage into the lung tissue. Of 30 mice given the acetins, no deaths occurred. None of the animals sacrificed on completion of injections showed significant changes in the lungs, heart, spleen, liver or kidneys. The growth of acetin injected rats and mice paralleled that of the uninjected controls.

The intraureteral injection of drugs into the renal pelvis of the dog. C. R. Linegar, Theodore Koppanyi and Melvin W. Green. *Dept. of Pharmacology and Materia Medica, Georgetown Univ., School of Medicine, Washington, D. C.* The injection of drugs into the lumen of the ureter by means of standard hypodermic syringe and needles produces responses comparable in speed and magnitude to those produced by intravenous injections. The volumes of the solutions injected varied between 1 and 5 cc. and at the completion of the injection the ureter was clamped and the disappearance of the liquid from the renal pelvis determined by the magnitude and duration of the pharmacological effect of the drug and in some cases by oncometric recording of kidney volume. Epinephrine (1–100,000 to 1–20,000), acetylcholine (1–100,000 to 1–10,000), pilocarpine, atropine and metrazol solutions in saline injected into the ureter in volumes up to 5 cc. produce hemodynamic and respiratory responses approximately as prompt and marked as if they were injected by intravenous route. The above drugs dissolved in 4 per cent polyvinyl alcohol and injected into the ureter are retained in the pelvis and produce no or slight effects only. Previous injection of polyvinyl alcohol into the ureter also prevents the effects of saline solutions of the above drugs injected via ureter. The rate of destruction of drugs in the kidney may be determined by clamping the renal vein before the intraureteral injection of a given drug and releasing the clamp at different intervals after injection. The more time elapses between the injection of the drug and the release of the clamp, the smaller the pharmacological effect of epinephrine or acetylcholine. The cause of this quick absorption of drugs from the pelvis is probably the pyelovenous reflux.

Bioassay of diuretics. Werner L. Lipschitz and Zareh Hadidian. *Lederle Laboratories, Pearl River, N. Y.* Male rats, weighing 140 to 240 grams and fasted for 18 hours were used. They were fed 25 ml./kgm. of 0.9 per cent NaCl at 37°C. containing the test substances. Sub-groups of four were placed on a funnel covered with metal mesh, inclosed by a glass cylinder and the urine from two sub-groups was drained into a volumetric cylinder. In all experiments one group of eight were fed isotonic sodium chloride solution; another, a given dose of urea, the standard diuretic; and the rest were fed various doses of the substance to be tested. The volume of urine excreted during the five hour period following feeding was expressed as percentage of the fluid administered, and the ratio of this percentage to the percentage excreted by the control group on the same day was taken as a measure of diuretic activity. A plot of activity as a function of log dose (millimoles kgm.) yielded straight lines of similar slopes in the case of urea, biuret, and various salts; and more complex curves in the case of the xanthine diuretics. The activity of urea was taken equal to unity and all other activities referred to this. The mean excretion in more than 100 control groups was 31 per cent of the fluid administered; while with high doses of some diuretics over 100 per cent was obtained. The following is a comparison of the relative diuretic activities found by the rat method with the relative activities (figures in parenthesis) calculated from human therapeutic doses: urea, 1, (1); sodium acetate, 2.5; potassium acetate, 3, (2.2); sodium nitrate, 3.5; potassium

nitrate, 4.5 (4–5); theobromine, 6–10, (230); caffein, 30–40, (400); theophylline, 100–150, (250); biuret, 1.5.

This method was found useful in assaying the activity of unknown diuretics.

Biochemorphology of the symmetrical ethers. DAVID FIELDING MARSH. *Univ. of California Medical School.* The inhalation toxicity, anesthetic potency, and explosibility of symmetrical ethers of two to ten total carbon atoms (dimethyl to diamyl ether) have been studied by methods previously described (Univ. Calif. Publ. Pharmacol. **1**: 369, 1940; **2**: 39, 1941). From the biological data it appears that the inhalation anesthetic power in the series varies inversely with volatility, until boiling points above 150°C. are reached, when the compounds concerned are not volatile enough to give an anesthetic concentration in air at room temperature. However, inhalation toxicity increases directly with molecular weight regardless of isomeric form. Hence, di-n-propyl ether, with a higher boiling point, is almost twice as safe an anesthetic agent as di-iso-propyl ether. Explosibility studies may be summarized by stating that the lower limit of inflammability in per cent in air or oxygen approximates the ratio of the integer six divided by the number of carbon atoms in the compound. The ratio of the lower limit of inflammability divided by anesthetic concentration varies directly with molecular weight and boiling point, and inversely with volatility. Force of explosion seems less with compounds of higher molecular weight. With di-n-propyl and higher ethers, it is necessary to exceed the LD_{50} concentration at 15 minutes inhalation in order to reach the lower limit of inflammability. The symmetrical ether optimum for inhalation anesthesia on the basis of these data seems to be di-n-propyl ether.

The action of cyclopropane and chloroform on the central origins of the sympathetic system. STEVENS J. MARTIN and AMEDEO S. MARRAZZI. *Depts. of Anesthesia and Pharmacology, New York Univ. College of Medicine.* The ability of cyclopropane and chloroform to cause cardiac irregularities, especially in conjunction with epinephrine, has been shown to be dependent in large part on suprapontine sympathetic centers (Allen, Stutzman and Meek; Beattie, Brow and Long).

Direct evidence as to whether the anaesthetics exercise their effects on the heart by modifying the activity of such centers has not been available. It has been obtained in these experiments by recording the sympathetic outflow indicated by action potentials in the preganglionic nerves.

Cats were used because of their great sensitivity to the cardiac effects of chloroform (Levy) and cyclopropane (Meek). Under these anaesthetics action potentials were recorded from the cervical sympathetic trunk and blood pressure from the femoral artery at each plane as the anaesthesia was progressively deepened and lightened.

Nembutal was then injected intraperitoneally in divided doses as the gases were washed out. After a period adequate for the almost complete elimination of the volatile anaesthetics records were again taken. Since nembutal does not cause cardiac irregularities (Meek), these records served as controls.

Comparison with the same animal's cyclopropane or chloroform record at the corresponding plane and blood pressure revealed no consistent significant difference, indicating lack of action on sympathetic centers. It is suggested that the functional integrity of sympathetic centers, requisite for eliciting chloroform or cyclopropane cardiac irregularities, is essential for maintaining responsiveness of the heart to the action of cyclopropane and chloroform which appears to be directly upon it. This interpretation is in accord with the views of Meek. [*Aided by a grant to A. S. M. from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association.*]

Toxicological studies on succinyl-sulfathiazole. PAUL A. MATTIS and A. D. WELCH. *Dept. of Pharmacology, Medical Research Division, Sharp and Dohme, Inc., Glenolden, Pa.* Succinyl-sulfathiazole, one of a series of sulfonamides synthesized by Moore, et al. and found to be poorly absorbed from the gastro-intestinal tract, has been shown (Poth and Knotts. Proc. Soc. Exper. Biol. Med. **48**: 129, 1941) to exert a marked coliform bacteriostatic action in the intestinal tract of dogs without causing toxic manifestations. Similar results were obtained in 40 humans.

Six monkeys were given a neutral solution of sodium succinyl-sulfathiazole by stomach tube every 4 hours for 30 days. Dosage, two monkeys per dose level, was 0.5, 1.5 and 5.0 grams per kilogram per day, respectively; two control monkeys received water concurrently. All animals gained weight and no toxic manifestations or histopathological changes were seen; attention was given to hematology, plasma protein, isoiodeikon excretion, kidney function, and drug concentration in blood and urine. A maximum of 1.3 mgm. per cent sulfathiazole and 5.3 mgm. per cent succinyl-sulfathiazole was found in the blood; urine studies indicated that less than 4 per cent of ingested drug was eliminated by the kidney.

The effect of high blood concentrations was studied in five of six unilaterally nephrectomized monkeys; pre-treatment kidney and liver were examined histologically. Parenteral injections of sodium succinyl-sulfathiazole (25 per cent solution), one gram per kilogram per day, were given

by various routes for 10 days. Blood levels reached 4.8 mgm. per cent sulfathiazole and 170 mgm. per cent succinyl-sulfathiazole; maximum urine concentration was 12.1 grams per cent. No toxic manifestation except temporary depression was seen. Histological studies showed moderate liver and kidney changes in some animals, although plasma protein concentration and isoiodeikon excretion was normal; moderate anemia was produced.

The toxicity of 1,2-dichloroethane, 2-chloro-2-butene and 1,2,3-trichlorobutane. ELTON L. MCCAWLEY. *Univ. of California Medical School.* The toxicity of the proposed industrial solvents 2-chloro-2-butene and 1,2,3-trichlorobutane is being studied in comparison with 1,2-dichloroethane. The latter kills mice within 30 minutes as a result of inhaling a vapor concentration of 0.25 mM/L of air. This is less than one tenth of the vapor saturation at room temperatures. Death may occur without preliminary anesthesia. Sufficient liver and kidney damage is produced after single sublethal exposures to result in death on a second or third exposure on subsequent days. 2-Chloro-2-butene is dangerous to mice on 30 minutes inhalation of 2.0 mM/L of air. At this concentration, anesthesia occurs after 2 to 3 minutes. Daily inhalations for 30 minutes of sublethal concentrations of 2-chloro-2-butene are much better tolerated than is the case with 1,2-dichloroethane. Symptoms of toxicity from the inhalation of vapors of 1,2,3-trichlorobutane occur only after several exposures for 30 minutes at daily intervals to atmospheres saturated with the solvent at room temperatures. However, this compound produces local irritation more readily than the others when placed directly in contact with the skin.

Polarographic investigation of local anesthetics. A. R. MCINTYRE and RAY E. KING. *Dept. of Physiology and Pharmacy; Univ. of Nebraska College of Medicine, Omaha.* Polarographic investigations of local anesthetics in phosphate buffer solutions reveals significant differences in $E_{1/2}$, n, i_d. The approximate values of these are as follows. The half-wave potentials for Pontocain, Procain and Intracain at a pH of 6.91 and in a concentration of approximately 0.2 millimols are —1.65, —1.68, and —1.75 respectively. Nupercain exhibits two waves at a pH of 6.34 and a concentration of 0.3 millimols. The half-wave potentials are —1.0 and —1.5. The values of id/m.mols quotients for Pontocain, Procain and Intracain at the above pH and concentration are respectively 5500, 1800, and 400. For Nupercain the two id/millimol values are 857 and 2571. "n" for Nupercain is 2 for the more positive wave and 1 for the more negative wave. For the other three anesthetics "n" is unity.

Pontocain exhibits a marked catalytic hydrogen wave. Other anesthetics so far examined do not exhibit catalytic hydrogen waves. At a concentration of 0.1 millimol the catalytic wave is about 20 times as great as the "normal" waves for the same concentration of other anesthetics examined. The Pontocain hydrogen catalytic wave increases with more concentrated solutions and is not suppressible with concentrations greater than 1 millimol.

$E_{1/2}$ for Nupercain varies markedly with the pH. The more acid the solution the more positive the potential. Reduction of Nupercain occurs in two steps. The less negative curve is about one third the height of the more negative curve. Maxima were shown by all anesthetics tested. The least maxima were shown by the two Nupercain waves. Intracain has a rounded maximum. Procain has a well defined, easily suppressible peak.

Chemotherapeutic effectiveness of certain sulfonamide, organic antimony, arsenic, and bismuth compounds in experimental Chagas' disease. BARBARA MCIVOR and HAMILTON H. ANDERSON. F. P. LUDUENA and C. D. LEAKE. *Univ. of California Medical School and Peiping Union Medical College.* A series of representative organic arsenicals, antimonials, and bismuth compounds are under study in comparison with various sulfonamides in mice inoculated intraperitoneally with a virulent strain of *Trypanosoma cruci*. The organism is in the blood by the seventh day after inoculation, and 94 per cent of infected animals die within 40 days after inoculation. Drugs studied were administered intraperitoneally at a dosage of one-fifth the LD_{50} on alternate days for five administrations. Treatment was started 7 days after inoculation. Most of the ordinary representatives of the types of drugs mentioned are inactive in prolonging the lives of infected mice or of clearing the blood stream of the organism. However, 60 days after inoculation there survived 3/20 of those treated with sulfathiazole, 5/20 with sobisminol, 4/20 with antimony catechol tri-isopropanolamine, 4/20 with trisodium antimony trithioglycolate, 3/20 with 5-arseno-7-iodo-8-hydroxyquinoline, 6/20 with 5-arseno-8-hydroxyquinoline, 11/20 with sodium bismuth thioglycollate, 12/20 with sulfacridine (F. Proescher), and 13/20 with N,N′disodium-dextrose-sulfonate-4,4′-diaminodiphenylsulfone. In no case was a cure affected, since Donovan bodies were uniformly found in heart muscle on sacrifice of the survivors.

Adrenal gland: Sensitivity of the X-zone to various hormonal substances. M. K. MCPHAIL and H. C. READ. *Dept. of Pharmacology, Dalhousie Univ., N. S.* A.P.L. and P.M.S. hormones caused degeneration of the X-zone of the adrenal

gland in intact immature male and female mice. That they act via the gonads is shown by their complete inactivity in the castrate. Similar results were obtained with urine of pregnancy (human). A.P.L. was without effect in the adult female.

Oestrone in the immature male and female caused a rapid disappearance of the X-zone. In the adult female it produces a ring of degeneration in the cortex between the X-zone and the zona fasiculata, the latter being predominately involved. In the adult male the ring of degeneration borders the medulla (the X-zone being absent).

Administration of progesterone and desoxycorticosterone acetate were without effect.

Gradual regeneration of the X-zone occurred following its destruction by the above mentioned substances.

New methods in experimental pharmacology. WALTER L. MENDENHALL. *Dept. of Pharmacology, Boston Univ. School of Medicine, Boston, Mass.* (Demonstration.) The following apparatus has been devised for pharmacological study:

1. A thoracic window to make direct observations on heart and lungs
2. An abdominal window to view intestinal movements and changes in appearance of blood vessels
3. A pleural cannula to observe changes in respiration
4. A modified Brodie bellows recorder to show volume changes

The use of the spinal cat for the estimation of the potency of cardiac glycosides. RAFAEL MENDEZ. *Dept. of Pharmacology, Harvard Medical School, Boston, Mass.* Macdonald (Quart. J. Pharm. Pharmacol. **3:** 450, 1930; **7:** 182, 1934) claimed that the variation among cats used in the estimation of the potency of a digitalis substance is smaller when the spinal cat is used than when the estimation is made with cats under ether anesthesia; and that the spinal cat is less sensitive to the lethal cardiac action of g-strophanthin.

We have confirmed Macdonald's results. In comparing the activity of different cardiac glycosides by both methods we have found that the increase in the resistance of spinal cats to the poisonous cardiac action is not uniform for the different glycosides. For example: while in the case of Digitoxin the lethal dose for the spinal cat is 174 per cent of that of the etherized cat, in the case of the Lanatosides B and C it is of the order of 130 per cent. This suggests that there must be extracardiac changes which, affecting certain areas in the nervous system, are capable of influencing the cardiac activity of different cardiac glycosides in a different way.

The action of veratridine upon the heart-lung preparation. GORDON K. MOE. *Dept. of Pharmacology, Harvard Medical School, Boston, Mass.* Recent studies from this laboratory have shown that spontaneous or artificially induced failure in the dog heart-lung preparation can be resolved by small doses (0.2–0.3 mgm.) of veratrine. These studies are being extended to various pure alkaloids of the veratrine complex.

The effects of veratridine are qualitatively similar to the action of the veratrine complex, and are demonstrable only when a state of failure exists. Effective doses cause a rapid fall in venous pressure, accompanied by a decrease in diastolic volume and an increased minute output. Increased inflow, accomplished by elevation of the venous reservoir, produces an increased systemic output with much lower increments of venous pressure after veratridine has been given. Coronary flow, recorded from a Morawitz cannula, increases temporarily. Despite the increased output of the right ventricle, the pressure recorded from a pulmonary arterial branch falls markedly, indicating pulmonary vasodilatation.

A perceptible response may follow doses as small as 0.05 mgm., but strong effects, lasting 45 to 60 minutes, are produced by 0.2 mgm. The "therapeutic" response to repeated small doses decreases in magnitude and duration. Irregularities followed by ventricular fibrillation are produced by larger doses, on one occasion by three doses of 0.2 mgm. each, given over a period of 50 minutes. Veratridine is roughly equivalent to veratrine in "therapeutic" potency, but is more potent in its toxic action on the heart-lung preparation.

The characteristic cardiac action is also exhibited by the base, cevine, of which veratridine is the veratric acid ester, but only with relatively huge doses (70 mgm.).

Biochemorphology of halogenated fatty acids. JAMES L. MORRISON. *Pharmacology Labs. of the Univ. of California Medical School, and the West Virginia Univ. School of Medicine.* A preliminary pharmacological comparison of certain halogenated fatty acids, containing one to six carbon atoms, with the corresponding fatty acids, indicates that 1, the oral toxicity for mice of the neutralized solutions of these acids decreases with lengthening of the carbon chain; 2, the inhibiting action of halogenated fatty acids on yeast fermentation increases with length of the straight carbon chain and with increase in atomic weight of the substituted halogen; 3, yeast inhibition is decreased by substitution of halogen on the β-carbon, by substitution of more than one halogen on any carbon, and by branching of the carbon chain, and 4, certain members of the series with a low dissociation constant are toxic for yeast at acid pH, but progressively lose toxicity as the pH approaches 7.

The toxicity, absorption and diuretic effect of a mercurated allylsuccinylurea with theophylline. P. A. NUHFER, C. H. MELLISH and J. A. BUCHTER. *The Lakeside Labs., Inc., Milwaukee, Wis.* The toxicity of a solution containing a mercurated allylsuccinylurea and theophylline in molar proportions of 1:1.20 was determined in rabbits and dogs and compared with that of Salyrgan-theophylline. In all cases each dose of the drugs was given intravenously to twelve rabbits and ten dogs and the animals were observed six weeks. In rabbits the $L.D_{50}$. for mercurated allylsuccinylurea with theophylline was 0.15 cc. (6 mgm. Hg)/kgm. This dose was tolerated by the dogs and ninety per cent survived when given 10 mgm. Hg/kgm. The $L.D_{50}$ in rabbits for Salyrgan-theophylline was 0.10 cc. (4 mgm. Hg)/kgm. and in dogs 10 mgm. Hg/kgm. was ninety per cent fatal. Subcutaneously in mice, the $L.D_{50}$.–24 hours for both drugs was an amount containing 0.08–0.10 mgm. Hg/gram.

The rate of absorption of mercury from the rabbit muscle was roughly proportional to the amount of theophylline in the solution containing the mercurated allylsuccinyl-urea. For example, approximately ninety-seven per cent was absorbed by one hour when the mercury-theophylline ratio was 1–1.20. Following administration of the theophylline-free preparation, ninety-six per cent of the mercury was absorbed by twenty-four hours, but only fifty-five per cent at four hours.

Electrocardiograms of unanesthetized dogs and blood pressure determinations in dogs anesthetized with pentobarbital sodium showed that in doses of 4.75 mgm. Hg/kgm. neither drug affected cardiac automaticity nor did serious change in blood pressure result from administration of 5.32 mgm. Hg/kgm.

In dogs, mercurated allylsuccinylurea elicited a marked diuretic response and increased excretion of sodium chloride in doses from 0.6 to 4.8 mgm. Hg/kgm.

Effect of a monopiperidine biuret and other hypnotics on the oxygen consumption of rat liver. SHIH-YI P'AN and CH'ING-SHAN LU. *Peiping Union Medical College.* The direct manometric method of Warburg was employed to study the effect of 1-diethylacetyl-5-5-cyclopentamethylene biuret, sodium barbital, sodium pentobarbital, and paraldehyde on the oxygen uptake of rat liver at 38°C. In concentrations of 0.1 gram per 100 cc., pentobarbital and the piperidine biuret depress the total oxygen uptake of rat liver during the first 60 minutes by 75 and 35 per cent respectively, while barbital and paraldehyde exert little or no inhibitory action during the same interval.

The pharmacological action of hydroxyethylapocupreine on some isolated tissues. GEORGE J. PASTORIUS. *Dept. of Physiology and Pharmacology, Univ. of Pittsburgh School of Medicine, Pittsburgh, Pa.* Hydroxyethylapocupreine dihydrochloride was compared with quinine hydrochloride and quinidine sulfate on isolated terrapin auricles, terrapin ventricular strips, and isolated intestinal strips of rabbit. Its vasoconstrictive properties were investigated by perfusion of the vascular system of the frog.

The contractions of isolated terrapin auricles were slightly slowed and slightly weakened when 0.1 per cent hydroxyethylapocupreine in Ringer's solution (buffered to pH 7.3) was applied, but recoveries in undrugged Ringer's solution were prompt. Weaker concentrations of hydroxyethylapocupreine produced no demonstrable change. Like concentrations of quinine and quinidine caused marked slowing and weakening of contractions, and usually damaged the preparations irreparably.

The contractions of terrapin ventricular strips were not demonstrably altered on application of buffered 0.1 per cent hydroxyethylapocupreine in Ringer's solution. Quinine and quinidine in the same concentration produced marked slowing and weakening of contractions.

Rhythmically contracting isolated intestinal strips of rabbit were slightly slowed and the contractions weakened by 0.005 to 0.01 per cent hydroxyethylapocupreine in blood-Locke's mixture. Weaker concentrations were without demonstrable effect. Like concentrations of quinine and quinidine invariably slowed and weakened the contractions, and many strips responded similarly to weaker concentrations, as 0.001 per cent.

Constant pressure perfusion of the frog vascular system with 0.01 per cent hydroxyethylapocupreine in Ringer's solution caused an average flow reduction of 8 per cent; the same concentration of quinine caused an average flow reduction of 22 per cent. With solutions of greater strength, quinine vasoconstriction was always more severe, although the differences in flow reduction were less marked.

On the mode of action of toxic doses of hydroxyethylapocupreine. GEORGE J. PASTORIUS and PAUL L. MCLAIN. *Dept. of Physiology and Pharmacology, Univ. of Pittsburgh School of Medicine, Pittsburgh, Pa.* The toxicity of hydroxyethylapocupreine is centered upon respiration, and death results from respiratory paralysis while circulation is yet well maintained. To determine the point of attack of the drug upon the respiratory mechanisms, four experiments were performed on rabbits anesthetized by pentobarbital-sodium. Records were made of the contractions of the freed diaphragm slip, of intratracheal pressures, and of blood pressures. The thresholds for single response and for tetanus of the diaphragm by phrenic nerve

faradization were determined. Hydroxyethylapocupreine dihydrochloride, 1.0 per cent solution, was injected intravenously at constant rates, (which were varied in the several experiments from 4 to 6 mgm./kilo/min.), to the point of respiratory paralysis. In each case, single or tetanic response of the diaphragm by nerve faradizations of threshold magnitude could be elicited throughout the injection period and for several minutes following cessation of respiration. Moreover, despite progressive diminution, and eventually cessation, of spontaneous diaphragm activity, the magnitude of response to single or multiple faradization of the nerve was never less than that of the control response.

Since the peripheral mechanisms remain intact, the respiratory depression produced by toxic doses of hydroxyethylapocupreine must, by inference, be due to action on the central mechanisms, and most probably, on the respiratory center itself.

Biological investigations with radioactive calcium and strontium. CHARLES PECHER (1912–1941), JOHN H. LAWRENCE and JOSEPH G. HAMILTON *Univ. of California Radiation Laboratory and Medical School.* Twenty-four hours after intravenous injection of salts of Ca^{45} and Sr^{89} in mice and rats, the concentration in bones (especially where new bone or dentine is forming) is about 100 times as much as in soft tissues, as estimated by radio-autography. Comparison of radiation distribution after oral or intravenous administration indicates that about one third of whatever calcium lactate is given by mouth may be absorbed. Comparison of distribution of radioactive strontium chloride, lactate, and gluconate after oral administration shows that intestinal absorption and osseous uptake of strontium is independent of the anion. Intravenously injected radioactive calcium or strontium lactate is excreted both in the urine and feces.

In patients with metastatic bone cancer, Sr^{89} has been given intravenously in the form of the lactate in a concentration of 20 mgm. per cc., with a total dose of radioactivity not exceeding 10 millicuries. This dosage may cause a transient leucopenia. Since the half-life of Sr^{89} is 55 days, a single administration has been enough to show in some patients effects upon which clinical judgment may be expressed.

The effect of dinitrophenol and thyroxin on the duration of sodium pentabarbital anesthesia in rats. S. A. PEOPLES. *Dept. of Physiology and Pharmacology, School of Medicine, Univ. of Alabama.* The rate of oxygen uptake of a group of fasting rats was determined and the duration of anesthesia produced by 30 mgm/kgm. of sodium pentabarbital given intraperitoneally was then ascertained. Thyroxin, 1 mgm/kgm., was then given daily by intraperitoneal injection for 3 days and on the fourth day the rate of oxygen uptake and duration of sodium pentabarbital anesthesia was again determined. There was an average increase in oxygen uptake of 65 per cent and the length of anesthesia was shortened by 31 per cent.

The duration of anesthesia was determined with a similar group of rats where the 30 mgm/kgm. of sodium pentabarbital was immediately followed by the subcutaneous injection of 10 mgm-kgm. of sodium dinitrophenol. This dose of sodium dinitrophenol had been found to cause a 60 per cent increase in the oxygen uptake over a period of 2 hours. This procedure shortened the anesthesia by 9 per cent, which is well within experimental error.

This evidence indicates that the shortening of sodium pentabarbital anesthesia is not due to a general increase in cellular metabolism but to a specific stimulant action on the central nervous system.

Ergotamine tartrate on work capacity of human muscle. CARL PFEIFFER and GEORGE L. MAISON. *Wayne Univ., College of Medicine, Detroit, Mich.* Because many migraine patients develop muscle pains and weakness after therapeutic doses of ergotamine tartrate (E.T.) and because these extreme symptoms may be due to the combination of migraine and E.T. or to the E.T. alone, a study of the work output in normal human subjects after E.T. was undertaken. Since E.T. produces a slight rise in blood pressure, small control doses of neosynephrin HCl were used to produce a comparable rise. Seven thoroughly trained (30 minutes daily for 3 to 24 months) subjects used an extensor ergograph (A.J.P. **132**, 390 (1941)) with a rapid work rate of 12 kgm.-cm./sec. The work ability was tested 90 minutes after $\frac{1}{4}$ to $\frac{3}{4}$ mgm. doses of E.T. and 15 minutes after 4 to 10 mgm. doses of neosynephrin HCl. Occasional placebo injections were interspersed without the recipient's knowledge. In 17 trials on the 7 subjects E.T. produced only occasional symptoms (nausea, masseter trismus, dizziness, sweating), and the work ability was normal or decreased (max. decrease 25 per cent). In 7 trials on 6 subjects neosynephrin HCl produced occasional symptoms (pilomotor activity, headache), and the work ability was normal or increased.

Since E.T. decreases slightly, if at all, the work capacity of muscle of normal, trained men, it seems unlikely that the muscle weakness reported clinically in treated migraine patients can be due to the direct action of E.T. Some predisposing migraine factor seems indicated. [*Aided by a grant from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association.*]

The antagonism between nicotine and physostigmine. ALBERT J. PLUMMER. *Dept. of Pharmacology, Boston Univ. School of Medicine, Boston, Mass.* Physostigmine is effective in overcoming the depression caused by nicotine in the frog and protects this animal against several lethal doses of the drug. This reaction is being analyzed by "in vitro" experiments and by mammalian studies in order to determine the site of action. The value of physostigmine as an effective antagonist in nicotine poisoning is also being considered.

The nature of Heinz bodies and their relation to sulfanilamide chanosis and anemia in mice. ARTHUR P. RICHARDSON. *Dept. of Pharmacology, Univ. of Tennessee Medical School, Memphis.* Within twenty-four hours after commencing administration of sulfanilamide, very small Heinz bodies appear in the majority of mature erythrocytes. These increase in size and by the fourth or fifth day when blood destruction is well established they reach diameter of about one to one and one-half micra. By controlling the degree of cyanosis with diets of varying concentrations of sulphur, it has been demonstrated that Heinz bodies occur whenever anemia is produced regardless of the presence or absence of changed blood pigment. On discontinuing sulfanilamide, Heinz Bodies persist in the blood for at least fourteen days. A method is described by which Heinz Bodies may be isolated in fairly pure form. Properties of these structures are described. Preliminary analyses indicate that they are made up of considerable amounts of cholesterol and protein but very little iron.

Studies on planta cundeamor. GILBERTO RIVERA. *Tropical School of Medicine, Univ. of Puerto Rico, under auspices of Columbia Univ., San Juan, P. R.* Planta Cundeamor, or Mormordica Charantia, is a plant long used by the natives of Puerto Rico for treatment of diabetes. Chemical studies resulted in the isolation of a crystalline substance having hypoglycemic effects on animals, and human cases of diabetes.

Preliminary studies on the pharmacology, toxicology, and hypoglycemic effect of the plant and the crystalline product are reported.

However, it is not recommended that this plant, or the crystalline substance isolated from it, be used for the treatment of diabetes in human cases until a more complete study of its toxicity and possible value has been made.

The influence of previous diet on the insulin tolerance of hypophysectomized rats. SIDNEY ROSENBERG and LEO T. SAMUELS. *Dept. of Physiology, Univ. of Minnesota, Minneapolis.* When hypophysectomized male rats were fasted 24–36 hours after being maintained on high fat diets by stomach tube, they showed as high a tolerance to intraperitoneal injections of insulin as did similarily fed controls. Controls fed an equicaloric high carbohydrate diet by stomach tube showed a similar insulin tolerance curve, but the carbohydrate-fed hypophysectomized rats showed the decreased tolerance which has been reported in such animals fed ad libitum.

Comparison of the data on blood sugars and carbo-hydrate stores indicates that the larger amount of fat available in the liver and circulation has a sparing effect on carbohydrate metabolism. The bearing of these observations on the general problem of the interreltions of carbohydrate and fat metabolism will be discussed. [*This investigation was supported by a grant from the Committee on Research in Endocrinology, National Research Council. Assistance in the preparation of these materials was furnished by the personnel of the Works Project Administration, Official Project no. 165-71-124, Sub-project no.* 355.]

The distribution of radioactive phosphorus in striated and cardiac muscle. JACOB SACKS and CHARLES H. ALTSHULER. *Dept. of Pharmacology, Univ. of Michigan Medical School, Ann Arbor.* Radioactive phosphorus (P^{32}) produced by bombardment of red P with deuterons, was converted to Na_2HPO_4 and injected subcutaneously into cats. The time course of the distribution among the acid-soluble phosphorus compounds of heart and of resting striated muscle was studied and correlated with the Pll content of the plasma inorganic phosphate.

It was found that the P^{32} content of the intracellular inorganic phosphate of the heart was very much greater than the corresponding value in resting striated muscle. The rates of new formation of phosphocreatine and adenosine triphosphate were found to be many times as great in heart as in the striated muscle. In the case of the hexosemonophosphate fraction, the difference between the two tissues was less marked.

The findings on P^{32} distribution btween plasma inorganic phosphate and the various P compounds of the intact beating heart are in good general agreement with those of Barker, Furchgott, and Shorr (Am. J. Physiol. **133:** P202, 1941) on resting slices in Ringer solution. From this it follows that the observed interchanges of phosphate groups are independent of the contraction process.

The results obtained are of value in elucidating the mech anism by which phosphate groups enter the muscle fiber, and in studying the inter-relations between the acid-soluble phosphorus compounds in the resting metabolism of striated muscle.

The work was supported by a grant from the John and Mary R. Markle Foundation.

The response of the rat uterus to sympathomimetic drugs. L. D. SEAGER, *Univ. of Tennessee.*

Isolated uterine segments from non pregnant rats were placed in Tyrode or Ringer-Locke solution in a Gibbs constant temperature bath, and the contractions mechanically recorded. Ephedrine sulfate, 1:1,000,000 to 1:5000, in 80 tests on 52 segments gave either inhibition or no change in all experiments. Benzedrine sulfate, 1:100,000 to 1:5000, in 80 tests on 30 segments produced increased amplitude tone or rate in 29 experiments, decreased activity in 31 and no change in 20. Propadrine, 1:500,000 to 1:5000, in 80 tests on 32 segments produced increased activity in 11 experiments, a decrease in 37 and no change in 32.

In over 200 tests with pinephrine, 1:150,000,000 to 1:5,000,000, on 80 segments either inhibition or no change was found. Synephrine and Neosynephrine in 20 experiments produced either inhibition or no change in activity.

Repeated small doses of ephedrine, propadrine or benzedrine usually resulted in refractoriness to further doses of the drug and refractoriness to the inhibitory effects of epinephrine and neosynephrine. Refractoriness was not developed by repeated small doses of epinephrine or neosynephrine.

Effects of subcutaneous injections of sodium tungstate on the rat. RAYMOND M. SELLE. *Dept. of Biology, Occidental College, Los Angeles, Calif.* Male rats which received daily subcutaneous doses of a .1 M aqueous solution of sodium tungstate at the rate of 0.05 cc per 10 grams of body weight weighed 26 per cent less than the controls, whereas their kidneys were 45 per cent heavier and their adrenal glands 43 per cent heavier than the controls. Likewise, the kidneys of the females which received the salt subcutaneously were 42 per cent heavier than those of the control females although their body weights averaged 11 per cent less. There were no differences between the weights of the bodies, kidneys and adrenal glands of male and female rats that received corresponding doses of the salt by stomach tube and their controls.

Sodium tungstate administered subcutaneously caused a severe drop in rectal temperature, from 100.7° to 92.5°F. in 2 hours. From then on the temperature rose slowly so that by 8 hours after the injection it had returned to 99.1°F.

Sodium tungstate administered by mouth was excreted in the urine and feces during the night and following day. When given subcutaneously most of the tungsten was eliminated in the urine within 12 hours.

Enzymatic deacetylation of acetylsulfonamide compounds. JAMES M. SHAFFER. *Dept. of Pharmacology, Univ. of Minnesota Medical School, Minneapolis.* A water soluble, heat labile, enzyme, or enzyme system, has been found in the chicken kidney, which will produce rapid deacetylation of acetylsulfanilamide, acetylsulfapyridine, acetylsulfathiazole, or acetylsulfadiazine in vitro. Either water suspensions of ground chicken kidney or dehydrated kidney preparations have been found to be effective. The activity of water suspensions of ground chicken kidney is inhibited to varying degrees by mercuric chloride, silver nitrate, chloral hydrate, sodium pentobarbital, sodium phenobarbital, quinine dihydrochloride, and soldium fluoride in concentrations of 0.001 M.

The deacetylating mechanism of the chicken kidney operates in the intact animal as evidenced by the finding that chickens receiving the four above mentioned drugs orally excrete the acetylsulfonamides in the urine largely in the free, unconjugated form. The degree to which these four acetylsulfonamides are converted to the free form in vivo varies inversely with their solubility in urine, i.e., acetylsulfathiazole and acetylsulfapyridine, the two least soluble, are converted to the free form to the greatest extent, while acetylsulfanilamide, the most soluble of the series, is converted to the smallest extent.

In a series of 28 chickens which had received one or more daily oral doses of either sulfanilamide, sulfapyridine, sulfathiazole, or sulfadiazine for periods of one to two weeks, analyses of kidney tissues showed the presence of a conjugated derivative in only 6 of the 28 chickens. In those 6 chickens the per cent of acetylation found in the kidney was much less than the per cent of acetylation found in the blood or liver.

Central stimulation studies on a number of amines. R. S. SHELTON, M. R. WARREN, T. J. BECKER and M. G. VAN CAMPEN. *Research Labs., The Wm. S. Merrell Company.* Recently Schulte et al (J. Pharmacol. and Exper. Therap. **71**: 62 (1941) reported studies on the central stimulant action of a series of sympathomimetic amines. The present investigation is an extension of this study. A total of 27 amines has been investigated for their central stimulant action. The compounds were variations of aryl-ethyl, aryl-phenyl, or aryl-butyl amine structures. The racemic hydrochlorides were employed unless otherwise stated.

The method for determining the central stimulant action was the same as that employed by Schulte and his coworkers. Male rats weighing between 200 and 250 grams were used for all tests. Twelve rats were used for each compound. The control value is based on an average of 200 animals. All compounds were administered orally at a dose of 50 mgm. per kgm. The tests were run for a period of six hours following the administration of the compound. The number of revolutions of the work-adder per hour was taken from the kymograph record, averaged, and compared with the control value.

An attempt has been made to correlate the effect of the various compounds with the chemical struc-

ture. dl-Phenylisopropylamine which was included in this series produced the optimum central stimulation. The results obtained with this series of amines indicate that the most effective molecular arrangement was phenyl-1-amino-2-propane and are therefore in agreement with those of Schulte et al. Any deviation from this configuration decreased this activity or abolished it entirely.

The effect of chronic morphine poisoning on the oxygen uptake of dog skeletal muscle. F. E. SHIDEMAN and M. H. SEEVERS. *Dept. of Pharmacology, Univ. of Wisconsin, Madison.* Specimens of muscle were obtained by sterile biopsy from the biceps femoris of nine normal dogs anesthetized with intravenous pentobarbital or procaine block of the sciatic nerve. The no-substrate oxygen uptake was measured over a period of 3 hours in the Warburg apparatus after mincing in a micromincer (Science **94**: 351, 1941). The following mean values for Q_{O_2} were obtained: left leg, 5.65 (4.49-7.00); right leg, 5.96 (4.47-7.37). Variation between right and left leg in a single animal was small even though several weeks intervened between determinations. The type of anesthesia did not influence the result.

Morphine (0.12 per cent) added *in vitro* has no constant effect on the oxygen uptake of dog skeletal muscle during the first hour but the rate of oxygen uptake during the second and third hours is increased so that the mean 3-hour U_{G_2}(15 experiments) is 16 per cent above the control level.

After these preliminary studies the daily administration of morphine (5 mgm. per kgm.) was begun and increased as tolerated. Oxygen uptake determinations were made on muscle from the left leg of six of these dogs now receiving 50 to 70 mgm. per kgm. after two to two and one-half months. A significant increase in the no-substrate oxygen consumption above the previously established control value for the same leg was observed in four of six animals after 48 or 72 hours of withdrawal. These preliminary results are similar to those previously obtained on chronically morphinized rats.

The *in vitro* addition of morphine to chronically morphinized dog muscle induces an effect which is qualitatively similar to that described above for normal muscle.

The oxygen consumption of cerebrum from chronically morphinized rats. F. E. SHIDEMAN and J. K. THEISEN. *Dept. of Pharmacology, Univ. of Wisconsin, Madison.* It has been demonstrated in this laboratory that skeletal muscle removed from chronically morphinized rats during withdrawal utilizes oxygen at a much greater rate than muscle from normal rats. The following preliminary report deals with a similar study on brain. Albino rats were poisoned over a period of three months with ascending doses of morphine sulfate (20 to 240 mgm. per kgm.). At various periods of withdrawal the brains were removed, the cerebri minced, and oxygen uptake determinations made. The no-substrate Q_{O_2} of minced cerebrum of 27 normal rats varied from 5.34 to 6.86, with a mean value of 6.15. The mean Q_{O_2} of minced cerebrum from 18 chronically morphinized rats killed at 1, 24, 48, or 72 hours after the last dose was found to be 6.28 (range 5.15–7.73). If these animals are grouped with respect to withdrawal time, mean values of the same order are obtained.

Addition of morphine (0.12 per cent) *in vitro* produced no significant effect on normal or chronically morphinized cerebrum.

A comparison of these results indicates that the total oxygen uptake of minced cerebrum of chronically morphinized rats does not differ significantly from normal. This finding does not preclude the possibility of a differential effect of morphine on oxidations in certain specialized cell groups or on specific oxidative enzymes of the central nervous system.

Effect of alcohol on post-pituitary antidiuresis. HERBERT SILVETTE. *Dept. of Pharmacology, Univ. of Virginia, Charlottesville.* The inhibition of typical pituitary antidiuresis in rats by injection of anesthetic doses of Pentothal-sodium has been previously reported (J. Pharmacol. and Exper. Therap. **72**: 37, 1941). In this paper are presented data concerning the effects of narcotic doses of ethyl alcohol on the course of the antidiuresis produced by post-pituitary extract.

Narcotic doses of alcohol were administered intraperitoneally to rats previously injected with 10 cc./100 grams 0.2 per cent saline. This resulted, not in diuresis, but in marked inhibition of urine output, similar to, but more prolonged than that observed after the administration of Pentothal. This urinary inhibition lasted as long as the animals remained in a deeply narcotized state.

The administration of post-pituitary preparations to animals anesthetized and rendered antidiuretic by alcohol resulted in augmentation of the alcohol antidiuresis, indicating that the pituitary hormone still exerted its antidiuretic effect. This observation was in interesting contrast to the well-known fact that the usual antidiuretic effect of post-pituitary solution is not observed in animals anesthetized with barbiturates. The antidiuretic response to post-pituitary was noted whether the hormone was injected before or after the administration of alcohol. Both Post-Pituitary Solution (U.S.P. XI, 2.5 units per 100 grams) and Pitressin Tannate in Oil (Parke-Davis, 0.125 cc.) were employed. The antidiuresis observed after the latter was profound, lasting for at least 24 hours, even in the presence of the large water-load employed in these experiments.

The effects of other narcotics on the course of pituitary antidiuresis are being investigated. [*This*

investigation has been made with the assistance of a grant from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association.]

The pain threshold response to morphine in humans as modified by prostigmin methylsulfate. DONALD SLAUGHTER and J. W. GALES. *Dept. of Physiology and Pharmacology, Baylor Univ. College of Medicine.* We have previously reported (J. Pharmacol. and Exper. Therap. **68**: 104, 1940; J.A.M.A. **115**: 2058, 1940) that prostigmin methylsulfate enhances the action of morphine on pressure-pain in cats, and that clinically it augments the relief of pain by morphine in humans.

This report deals with preliminary experiments on seven normal humans performed on a modified Wolff-Hardy-Goodell quantitative, analgetic machine.

Results show that the average increased temperature thresholds were as follows: 8.3°C. with 16 mg. morphine sulfate; 1.4°C. with 8 mgm. morphine sulfate; and 5.2°C. with 8 mg. morphine sulfate + 0.5 mgm. of "prostigmin".

The above doses raised the threshold temperature over the normal value 23.4°, 9.5° and 12.8°C, respectively.

When the time in which the peak threshold was reached is compared, there is very little difference between the small and large dose of morphine and the small dose of morphine + "prostigmin."

As regards the total time of effect, the small dose of morphine + "prostigmin" is essentially the same as the large dose of morphine alone; i.e., 247 and 243 minutes, respectively. By contrast, the small dose of morphine alone lasted approximately one hour less than either of the above (183 min.).

These results indicate that "prostigmin" enhances the quantitative pain response to morphine and appears to corroborate our previous experimental and clinical reports.

The application of the photoelectric colorimeter for the determination of ergot alkaloids. RALPH G. SMITH, *Laby. of Pharmacology, Univ. of Michigan Medical School, Ann Arbor.* By use of the Evelyn photoelectric colorimeter with a 550 mμ filter complete calibration curves have been prepared for ergotoxine and ergonovine, using solutions of ergonovine maleate (Eli Lilly & Co.) prepared as a standard for the U. S. Pharmacopoeia and of ergotoxine ethanesulfonate (Burroughs Wellcome & Co.). Expressed as mgm. per cc. of the alkaloidal bases, the concentrations covered by the curves extend from 0.098 to 0.00168 for ergotoxine and from 0.059 to 0.00064 for ergonovine. In each test there was used 4 cc. of the alkaloidal solution and 8 cc. of the Allport and Cocking modification of the paradimethylaminobenzaldehyde solution. Readings were made twenty minutes after the addition of the reagent. The photometric density varies closely with the concentration over limited portions of the curves. Between galvanometer readings 20 and 70 the calibration constant (K_1) is 18.9, S.D. ± 0.41 for ergotoxine and 33.2, S.D. ± 0.37 for ergonovine. Over more limited ranges less deviation was found since K_1 for both alkaloids decreases gradually with the concentration and falls rapidly at the higher dilutions. Using the above values for K_1 the color ratio of ergonovine:ergotoxine is 1.76 in contrast to the theoretical relationship of 1.87 based on the molecular weights. By comparison of the curves this value varies inversely with the concentration from 1.67 to 1.95. In assaying these alkaloids, unless using dilutions within a limited range, calibration curves rather than constants should be used.

Effect of lowered oxygen tension of inspired air on the respiratory response of normal subjects to carbon dioxide. MAYO H. SOLEY and NATHAN W. SHOCK. *Univ. of California Medical School.* To ascertain whether or not a reduction in the oxygen tension of the inspired air would cause a decreased response to carbon dioxide, 18 male college students were each tested twice with accurate concentrations of these gases in air. Inhalation of 17 per cent oxygen causes no significant change in respiratory volume while 12 per cenr oxygen gives an average increment of 10.6 per cent Inhalation of 2 per cent carbon dioxide mixed with 21 per cent oxygen increases the respiratory volume 28 per cent, a figure that does not differ significantly from the increase of 34 per cent found previously or from that of 33 per cent caused by breathing 2 per cent carbon dioxide mixed with 17 per cent oxygen. Inhalation of 2 per cent carbon dioxide mixed with 12 per cent oxygen results in an increase of 38.9 per cent in respiratory volume which is significantly greater than the increase caused by inhalation of 2 per cent carbon dioxide in 21 per cent oxygen. Therefore it may be concluded that reduction of the oxygen content of inspired air to 17 per cent or even to 12 per cent at sea level does not decrease the respiratory response to carbon dioxide. On the contrary, a mixture of 2 per cent carbon dioxide and 12 per cent oxygen stimulates respiration more than a mixture of 2 per cent carbon dioxide and air.

Toxic range and reaction of 4,4′ diamidino stilbene and p-tolamidine hydrochloride. H. Y. SOONG and S. C. FU. *Peiping Union Medical College.* As part of a study of the chemotherapy of leishmaniasis (Am. J. Trop. Med. **21**: 461, 1941), the intraperitoneal toxicity of 4,4′ diamidino stilbene and p-tolamidine hydrochloride was determined in inbred white mice and stock Chinese hamsters (Cricetulus griseus). The LD_{50} of 4,4′ diamidino stilbene was 50 mgm. per kgm. for hamsters and from 43 to 53 mgm. per kgm. for mice, while the LD_{50} of p-tolamidine hydrochloride was 235 mgm.

per kgm. for mice and 150 mgm. per kgm. for hamsters. Small hamsters weighing from 10 to 14 grams died when given 25 mgm. per kgm. of the diamidine.

These findings would indicate that diamidino stilbene is more toxic than originally reported by Lourie and Yorke (Ann. Trop. Med. and Parasit. **33**: 289, 1939). Tolamidine was found to be ineffective against experimental leishmaniasis. Diamidino stilbene is now being studied in this connection.

A definite precipitation of 4,4′ diamidino stilbene occurs when it is added, in 1 per cent water solution, to serum, due presumably to the slight alkalinity of the blood or to a salting out process. Toxic reactions, including sudden deaths, reported in the literature may be accounted for by serum flocculation of proteins by the drug. [*4,4′ diamidino stilbene was supplied by Merck & C., Rahway, N. J. and p-tolamidine hydrochloride by Dr. Peter P. T. Sah and Parke, Davis & Co., Detroit, Mich.*]

Public health hazard of certain resins proposed for fruit coatings. SERGEI V. SPIRIDONOFF. *Univ. of California Medical School.* Mixtures of polymers of cumarone, indene, and other polymerizable constituents of coal tar naphthas have been proposed as protective coatings for certain fruits to prevent spoilage during transport and storage. On single oral administration to mice of a standardized mixture of this sort, the LD_{50} is 50 grams per kilogram with death due to intestinal blockage by this massive dose. On repeated daily oral administration to mice, 5 grams per kilogram kills half the animals within 5 days. There is no evidence of absorption of the resin, but its presence in the intestinal tract interferes with food utilization as indicated by progressive weight loss. However, no loss of weight occurs as a result of the daily oral feeding of 100 mgm./kgm. of the resin in olive oil to mice for three months, although the mortality is 10 per cent and associated with respiratory infection. The average amount of resin effective as a coating on an apple or orange is about 10 mgm. Daily oral administration of 10 mgm./kgm. of the resin to mice for five months results in no untoward effects at all, as far as can be determined.

Influence of phenolphthalein and sulfanilamide on the vitamin A distribution in the liver. FREDERICK STEIGMANN, HANS POPPER and HATTIE DYNIEWICZ. *Depts. of Pharmacology and Therapeutics and Internal Medicine, Univ. of Illinois College of Medicine and Cook County Hospital, and Cook County Graduate School of Medicine.* Prolonged clinical investigations with liver function tests, studies on monkeys and lifetime experiments on cats and mice did not show pathologic liver changes following ingestion of phenolphthalein.

Recently changes in content and distribution of vitamin A in the liver as seen under the fluorescence microscope have been used for demonstration of impaired liver function. These alterations are seen before anatomical changes are discerned. Hence, the effect of phenolphthalein on the liver when given alone in various percentages, when given to rats on different diets and when given in combination with sulfanilamide was studied by fluorescence microscopy.

There were no signs of liver disturbance in rats receiving 1 per cent phenolphthalein (white or yellow) comparable to a daily human dose of 43 grams. On 2 or 3 per cent of phenolphthalein slight to marked fatty changes respectively occurred in the liver, with inhomogeneous vitamin A distribution.

Of rats on different diets and receiving 2 per cent phenolphthalein (white or yellow), the least changes in vitamin A content and distribution were noted in those on high protein diet. Changes appeared in all animals after diets were continued for 7 weeks. White phenolphthalein seemed to cause less changes than yellow.

In rats receiving 2 per cent phenolphthalein (white and yellow), 1 per cent sulfanilamide and 1 per cent phenolphthalein (white and yellow), or 2 per cent sulfanilamide alone mixed with their food slight changes in vitamin A distribution were found after 30 days in those on sulfanilamide alone and on 2 per cent yellow phenolphthalein. With longer duration of the feeding and increase in the percentage of sulfanilamide and phenolphthalein, the changes became more marked.

Potential industrial hazard from "mouthing" galvanized nails. JOSEPH B. SWIM, ELTON L. MCCAWLEY and LOUIS A. STRAIT. *Univ. of California Medical School.* Shinglers and roofers, like lathers, are accustomed to "mouthing" nails. To increase resistance to weather, such nails may be galvanized (zinc-coated) either by a hot-dip process or electrolytically. The possible industrial hazard of long continued "mouthing" of such nails was studied by estimating the extent to which zinc might be dissolved by saliva (nil as found spectrographically), and the extent to which zinc might be converted to the locally irritating zinc chloride by gastric juice after swallowing zinc fragments or whole nails. The amount of zinc chloride formed from average quantities of strongly acid gastric juice and the total zinc coatings from ten galvanized shingle nails is too small to permit a concentration sufficient to cause local irritation of the stomach or intestinal tract. Since the zinc coating on a galvanized shingle nail averages about 80 milligrams, there is no danger of significantly disturbing the normal zinc balance of the human body, even if the coating were fully dissolved and absorbed. However, an alloy of tin and lead may be substituted for zinc in order to give a smooth

coating to nails of this sort. This is dangerous, and chronic lead poisoning may result from "mouthing" such nails.

A colorimetric method for estrone and estradiol. CLARA M. SZEGO and LEO T. SAMUELS. *Dept. of Physiology, Univ. of Minnesota, School of Medicine, Minneapolis.* A colorimetric method has been developed for the simultaneous determination of absolute amounts of pure estrone and estradiol in a single sample of a mixture. Guaiacolsulfonic acid in sulfuric acid solution is used as the color-producing reagent.

The concentrations of estrone and estradiol are calculated from the light absorption of the colored solution at 520μ and 440μ. The formation of a yellow colored compound by estradiol and the production of a pink compound by estrone appear to be independent of each other.

The reaction follows Beer's law for concentrations of estradiol through 50γ, and for estrone, through 30γ.

Estriol has no significant interference value. The following compounds to not give the reaction: progesterone, cholesterol, equilin, androsterone.

The following examples of some representative recoveries on single determinations indicate the accuracy of the method: In a sample containing 10γ estrone and 20γ estradiol, 9.6γ and 19.7γ were found, respectively. A calculated recovery of 4.9γ estrone and 10.1γ estradiol in another instance corresponds to 5γ estrone and 10γ estradiol actually present. From a sample of 5γ estrone and 2γ estradiol, the calculated recovery was 4.7γ and 2.27γ.

The accuracy of the method is increased when the calculations are based upon average figures from several determinations—e.g., in a known 15γ estrone-15γ estradiol mixture, the average values for triplicate determinations were 14.35γ and 15.2γ, respectively. [*Assistance in the preparation of these materials was furnished by the personnel of the Works Project Adminstration, Official Project no. 165-1-71-124, Sub-project no. 343*]

The coenzyme I content of liver and skeletal muscle of chronically morphinized rats. J. K. THEISEN and F. E. SHIDEMAN. *Dept. of Pharmacology, Univ. of Wisconsin, Madison.* Coenzyme I assays were made on liver and skeletal muscle from chronically morphinized rats during withdrawal by the method of Axelrod and Elvehjem. The values obtained in preliminary experiments were much lower than those found by these investigators for the normal rat. Further study revealed that the coenzyme I content of like tissues from presumably normal stock rats from our laboratory colony was even lower. Upon investigation it was ascertained that certain ingredients had been left out of the presumed adequate diet by the technician responsible for its preparation. After reviewing all of the assay values obtained on normal and chronically morphinized rats, which had been maintained on this deficient diet, it was apparent that the coenzyme I content of the liver and muscle was significantly higher in the chronically morphinized than in the unpoisoned stock rats. When an adequate diet was substituted for the deficient diet, the coenzyme I content of these two tissues from both normal and chronically morphinized rats was greatly increased. It has not yet been determined whether a higher coenzyme I content exists in tissues of chronically morphinized rats maintained on an adequate diet, but studies are now in progress to determine this point and will be reported.

Cutaneous vascular permeability and fragility in rabbits as influenced by lemon citrin. C. H. THIENES, GEORGE J. MAJOVSKI and CARL SCHUBERT. *Univ. of Southern California.* One group of rabbits was placed on the Sherman-La Mer scorbutogenic diet; controls received commercial rabbit diet. The fragility of the vessels of the shaved abdominal skin was tested at weekly intervals by means of a standardized vacuum cup, at 15, 20, 25 and 30 Cm. Hg suction for 1 minute. The number of petechiae increased over a period of 7 weeks, in the animals on the deficient diet, and the skin had a flushed appearance.

Permeability of the tissues was tested at the end of the 7 week period by measuring the rate of disappearance of cutaneous wheals produced by intracutaneous injections of 0.1 cc. physiological saline solution. The absorption time in controls varied from 60 to 90 minutes, but in the deficient animals the wheals disappeared in 20 to 30 minutes. At the end of 7 weeks, half of the deficient animals were given a supplement of crude lemon citrin in the drinking water. Fragility and permeability tests showed on these rabbits a return to normal values within 10 days, while the untreated group showed no change. [*Aided by Works Projects Administration project No. C.S.-12295 and by a grant from the California Fruit Growers Exchange.*]

The actions of ether and chloroform in relation to the choline esterase mechanism. CLARA TORDA. *Dept. of Pharmacology, Cornell Univ. Medical College, New York City.* Chloroform produces effects in the intact animal suggesting stimulation of the parasympathetic nervous system, but ether does not. This observation led to the investigation of the influence of these substances on choline esterase.

The results show that chloroform and ether inhibit choline esterase activity. These were secured in two types of experiments. It was found that ether and chloroform, in proportion to their concentration, inhibit the activity of choline esterase in tissue hash. It was also found that

low concentrations of chloroform (0.0025 to 0.03 vol. per cent) and ether (0.05 to 1.5 vol. per cent) increase the sensitivity of the isolated rectus abdominus muscle of the frog to acetylcholine. This effect is abolished by atropine or an excess of potassium chloride.

Higher concentrations of chloroform and ether depress the acetylcholine sensitivity of the muscle, and if sufficiently great, cause a contracture of the muscle (above 0.12 vol. per cent for chloroform and above 3.6 volumes per cent for ether). This contracture is not significantly influenced by atropine, acetylcholine, physostigmine or an excess of potassium chloride. These observations suggest that the effects of *high* concentrations of chloroform and ether are independent of influences on the choline esterase-acetylcholine mechanism and of processes relating to the liberation of potassium during muscle contraction.

It may be assumed that during anesthesia the neuromuscular system contains sufficient chloroform, but insufficient ether significantly to inhibit choline esterase.

A study of cardiac hemorrhages caused by the digitalis glycosides. JANET TRAVELL, HARRY GOLD, WALTER MODELL and OSCAR AUERBACH. *Dept. of Pharmacology, Cornell Univ. Medical College, and Sea View Hospital, New York City.* It is well known that the parenteral administration of the digitalis materials produces cardiac hemorrhages in animals. In view of the absence of evidence concerning the application of this phenomenon to man, we made post mortem examinations of the hearts of a series of patients who had received very large oral doses of digitalis for varying periods of time prior to death. These observations, in comparison with a control group, show that under these conditions digitalis does not cause cardiac hemorrhages in humans.

Using small groups of cats at different times, we have observed great variations in the incidence of cardiac hemorrhages by digitalis and various glycosides of the group given parenterally. We have investigated the role of certain factors which might influence the susceptibility of animals to these hemorrhages, such as the season of the year, special diets, vitamins, liver extracts, and the route of administration.

The most striking fact issuing from these experiments is that the incidence of cardiac hemorrhages after oral administration of the drug is negligible. That this lowered incidence is not due to the influence of the liver following absorption from the gastrointestinal tract is shown by the fact that portal and femoral vein injections gave the same high incidence of hemorrhages.

The effect of some organic solvents on the hormones of the posterior lobe of the hypophysis. JOHN A. VAICHULIS. *Loyola Univ. School of Medicine.* Fifty milligram quantities of U.S.P. posterior lobe powder were extracted with 20 cc. of the following solvents: methyl ethyl ketone, iso-propyl chloride, iso-butyl chloride, tertiary butyl chloride, iso-propyl ether (free of alcohols), and pentane. After twenty-four hours of extraction all of the original activity remained in the powder. When these powders were extracted for an additional twenty-four hours with mixtures of 5 cc. of the above solvents and 20 cc. of absolute methyl alcohol varying results were obtained. The alcohol mixtures of methyl ethyl ketone, iso-propyl chloride, iso-butyl chloride, and tertiary butyl chloride destroyed practically all of the activity. In the case of the iso-propyl ether and pentane alcoholic mixtures, the alcohol soluble fraction contained a preponderance of the oxytocic hormone, whereas the residual powder showed a 50 per cent decrease in the oxytocic hormone and less than 5 per cent decrease in the pressor hormone. At the present time longer periods of extraction are being carried out. [*Aided by a grant from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association.*]

Studies on the treatment of experimental carbon monoxide poisoning. W. F. VON OETTINGEN, D. D. DONAHUE, P. J. VALAER and J. W. MILLER. *Division of Industrial Hygiene, National Inst. of Health, Bethesda, Md.* Previously (W. F. von Oettingen, D. D. Donahue, P. J. Valaer and J. W. Miller. Public Health Bull. 274, U. S. Govt. Printing Office) it was shown that during and following exposure to carbon monoxide the spinal and intracranial pressures of dogs anesthetized with sodium amytal undergo certain changes which are paralleled by changes in the circulation. This study deals with the effect of inhalation of air, pure oxygen and a 6 per cent carbon dioxide-oxygen mixture on the spinal pressure, circulation and respiration of dogs poisoned with carbon monoxide until their blood contained approximately 60 per cent carbon monoxide hemoglobin. Inhalation of pure oxygen, or to a greater extent a 6 per cent carbon dioxide-oxygen mixture, for one-half hour favors the elimination of carbon monoxide from the blood, as compared with inhalation of air, but does not materially lower the spinal pressure. The arterial blood pressure is increased and the venous pressure decreased by inhalation of pure oxygen, whereas the inhalation of a 6 per cent carbon dioxide-oxygen mixture for one-half hour reduces the arterial pressure and increases the venous pressure. The latter treatment increases the respiratory volume, which effect outlasts the actual administration for several hours. Inhalation of pure oxygen has no such favorable effect. The experiments indicate that, following carbon monoxide poisoning, the circu-

lation and respiration may be unfavorably affected by administration of a 6 per cent carbon dioxide-oxygen mixture and pure oxygen, respectively.

Concerning the mechanism of membrane semipermeability. J. H. WEATHERBY. *Dept. of Physiology and Pharmacology, Medical College of Virginia, Richmond.* (*Demonstration.*) If lecithin and cephalin or either alone are dissolved in collodion solutions, and membranes prepared from this mixture, such membranes often exhibit asymmetry potentials of considerable magnitudes. These potentials vary with changes in pH. Apparently Lecithin contributes the positive component of the potential, which is most evident at relatively high hydrogen ion concentrations; and Cephalin contributes the negative component, which is evident at a hydrogen ion concentration of approximately pH 5 and above. Alkaloids such as nicotine permeate these membranes when they are negatively charged, but not when positively charged. Weak organic acids permeate the positively charged membrane, but not when it is negatively charged. The toxicity of nicotine solution at various pH values for living organisms (paramecia) approximately parallels permeability of artificial membranes containing Lecithin and Cephalin at these same pH values.

Succinyl-sulfathiazole as an inhibitor of bacterial syntheses in nutrition experiments. A. D. WELCH. (*Dept. of Pharmacology, Medical Research Division, Sharp and Dohme, Inc., Glenolden, Pa.*) When succinyl-sulfathiazole is added (5 per cent) to a commercial food, no toxic manifestations and no depression of growth rate occur in rats consuming the diet for 5 weeks. When added (1 per cent) to a diet of casein, sucrose, corn oil, salts and crystalline vitamins, a marked depression of growth rate is produced; further addition of p-aminobenzoic acid causes no favorable effect, whereas the addition of liver extract results in a growth rate comparable to that of the controls.

Black, McKibbin and Elvehjem (Proc. Soc. Exper. Biol. Med. **47**: 308, 1941 (obtained similar results with sulfaguanidine (0.5 per cent) except that p-aminobenzoic acid had considerable antagonistic action. These workers suggest an "inhibition of intestinal synthesis of essential growth factors which liver extract supplies, or . . . a toxicity which is counteracted by a factor in liver extract."

The results obtained with the relatively nontoxic succinyl-sulfathiazole (also Mattis and Welch, these Proceedings) seem best explained on the basis of bacterial synthesis of essential growth factors. The greater toxicity of sulfaguanidine (Black et al. found that many animals receiving 2 per cent died within 2 weeks) and the antagonism exerted by p-aminobenzoic acid suggest that sulfaguanidine interferes with syntheses of p-aminobenzoic acid and some unknown substance(s). The formula of N^4-succinyl-sulfathiazole and the lack of influence of p-aminobenzoic acid suggest that the influence of this drug is mediated through systems at present unknown. The nature and distribution of factors antagonizing the activity of succinyl-sulfathiazole as a growth-depressant in highly purified diets are under further investigation.

The effect of autonomic drugs on the contraction of the colon produced by pelvic nerve stimulation. J. A. WELLS and T. MERCER. *Dept. of Physiology and Pharmacology, Northwestern Univ. Medical School, Chicago, Ill.* The effect of autonomic drugs on the response of the urinary bladder to pelvic nerve stimulation has been studied and found to be complex (Henderson and Roepke. J. Pharm. and Exper. Therap. **54**: 408, 1935).

Similar studies on the response of the colon to pelvic nerve stimulation have not been found in the literature.

Electrical stimulation of the pelvic nerve in dogs produced a prompt and marked contraction of the longitudinal and circular muscle of the distal and descending colon with no effect on the proximal colon. This response could be duplicated by the intrarterial injection of acetyl choline, inhibited by intravenous atropine, and potentiated by subcutaneous physostigmine. The response was unaffected by intravenous ergotamine. It would appear, therefore, that acetyl choline is the substance which mediates the response of the colon to pelvic nerve stimulation.

Atropine does not separate the response of the colon into a contractile and a tonus mechanism as in the urinary bladder; however, it is more effective in inhibiting the response to pelvic nerve stimulation than to intravenous acetyl choline.

Serum cholinesterase activity in relation to morphine addiction in man. EDWIN G. WILLIAMS. *U. S. P. H. S. Hospital, Lexington, Ky.* Using the manometric method of Rinkel and Pijoan, determinations of cholinesterase activity were made on 70 normal controls, 32 patients during maintained addiction, 24 during withdrawal, and 37 post-addicts. In addition to these a number of studies were made on post-addicts before and after single injections of morphine.

The values for post-addicts and maintained addicts were higher than those for the control group. Patients during withdrawal showed definitely less cholinesterase activity than any other group studied. These changes were found to be statistically significant.

The cholinesterase activity 30 to 45 minutes following an injection of morphine was only slightly diminished with the larger doses (50 mgm.). Slaughter and Lackey observed a 20%

reduction in dogs after 5 mgm. of morphine per kilogram was given. The patients in this study were post-addicts, given smaller amounts of morphine per kilogram (approximately 0.7 mgm.).

The significance of these findings is discussed.

Cardiovascular effects of pituitary preparations which contain the oxytocic principle. R. A. Woodbury and W. F. Hamilton. *Dept. of Physiology and Pharmacology, Univ. of Georgia School of Medicine, Augusta.* As is well known, pituitary preparations which contain the oxytocic principle produce a marked and transient reduction in the arterial pressure of the fowl. As recently reported (Am. J. Physiol. **129:** 500, 1940), man shows a similar response to these preparations while only a small pressure reduction occurs in dogs and cats even though large doses are administered.

These studies have been extended and show that the order of increasing susceptibility is as follows: dog, cat, turtle, rabbit, chicken and man. Electrocardiograms, pressure pulses from the systemic and pulmonary arteries and data from the perfused heart are used in the analysis of the mechanism of the reduction of the arterial pressure. In some animals vasodilation seems to play a rôle. However, depression of the heart with little or no change in coronary flow is the important factor. In some experiments, the vagus ending appears to be stimulated even to the point of producing cardiac arrest.

This work was aided by a grant from the Josiah Macy Jr. Foundation.

The irritating properties of a propylene glycol solution of sulfanilamide. Frederick F. Yonkman, Frank Bruce, Marshall Purdy, Garnett Ice and John Jacobs. *Wayne Univ. College of Medicine, Detroit, Mich.* A 10 per cent solution (pH of 5.3 to 5.6) of sulfanilamide in propylene glycol was injected in either 10, 20, 25, 50 or 100 per cent concentrations in terms of propylene glycol content, diluted with 0.9 per cent sodium chloride, into rats, rabbits, dogs and man.

The subjects were observed for immediate and delayed effects and all pathologic studies were conducted by Dr. Mark Maun and Dr. Luverne Domeier of the Department of Pathology. Rats receiving 10, 20, 50 and 100 per cent propylene glycol solutions of sulfanilamide intraperitoneally, elicited with rare exception, no signs of discomfort or other unpleasant reactions. Autopsy examination, macroscopic and microscopic, after 24, 36 and 72 hours revealed, again with rare exceptions, no signs of inflammatory reactions. Unanesthetized dogs receiving solutions of 50 per cent concentrations of propylene glycol intraperitoneally were moderately uncomfortable for two or three minutes, either from peritoneal reactions or more probably from intramuscular or subcutaneous irritation attending minute residual deposition of propylene glycol upon withdrawal of the needle. Dogs under pentobarbital anesthesia in mid-Fowler's position to allow bathing of intestinal segments in the pelvic vault for one hour revealed varying degrees of hyperemia of the bathed segments but no signs whatever of irritation or inflammation on microscopic examination and all of our laparotomized dogs now routinely receive from one to two ounces of sulfanilamide propylene glycol solutions diluted 1:1 with saline intraperitoneally as a prophylactic. Rabbits, dogs and human subjects presented no immediate or delayed evidence of venous irritation after intravenous injection of saline dilutions of the stock solution with a propylene glycol content of 10, 20 and 50 per cent in rabbits, 25 and 50 per cent in dogs and 25 per cent in man. Intramuscular injection of 1 cc. of the stock solution diluted 1:1 with saline or Locke's solution were transiently irritating for from one-half to one minute in human subjects but no residual soreness prevailed beyond the first hour. The immediate discomfort was significantly greater than that attending control injections of sterile saline and although irritation was generally not so severe as to militate against this route of injection in real emergency such as prevails in a vomiting patient with sclerosed veins, one would not choose the intramuscular route as that of first choice. The stock solution of 10 per cent sulfanilamide in 100 per cent propylene glycol elicited no signs or symptoms of irritation or discomfort when applied to freshly scarified areas on the forearms of the authors.

Propylene glycol as a solvent for sulfonamides. Frederick F. Yonkman, A. J. Lehman and H. F. Chase. *Wayne Univ. College of Medicine, Detroit, Mich.* Propylene glycol serves as a medium in which one can dissolve 10 per cent of sulfanilamide, 3 per cent of sulfathiazole, 3 per cent of sulfapyridine and approximately 0.3 per cent of sulfadiazine. These solutions are made over a water bath at between 60 and 80°C. for from ten to twenty minutes and persist if preserved at a temperature of 35°C. or more.

Some samples are now six months old and are apparently unchanged. Prolonged cooling will result in varying degrees of crystallization which disappears on moderate reheating in a water bath or over a warm radiator overnight. Solutions of sulfanilamide and sulfathiazole have been studied in considerable detail and when thus treated retain their potency, are self-sterilizing (Joslin and Hasley) and can be diluted with saline before using without change in homogeneity of the solution. In contradistinction to ethylene and diethylene glycol, propylene glycol is practically non toxic even in considerable amounts and the advantages of a concentrated, self-sterilizing, bac-

tericidal, dilutable and stable solution of certain sulfonamides warrant further study of this type of preparation.

Blood levels of sulfanilamide following intraperitoneal injection of a 10 per cent solution of sulfanilamide in propylene glycol. FREDERICK F. YONKMAN and CLARENCE WALTON. *Wayne Univ. College of Medicine, Detroit, Mich.* Of the numerous dogs receiving varying amounts of the so called 'all purpose' stock solution of 10 per cent sulfanilamide in propylene glycol intraperitoneally, six have been studied for plasma levels of free sulfanilamide at definite intervals after parenteral insertion of a 50 per cent saline dilution. Results are summarized: *a.* In two dogs under pentobarbital anesthesia, one hour after insertion of from 20 to 30 cc. of stock solution, respective blood levels were 35 and 26 mgm. per cent.

b. In one unanesthetized dog, insertion of fluid representing 10 mgm. of SMD per kg. produced blood levels of 2 mgm. after 15 minutes, 1.1 mgm. in 90 minutes and 0.8 mgm. in four hours; in the other, injection of fluid equivalent to 20 mgm. of SMD per kg. produced blood levels of 2.5 mgm. in 30 minutes, 2.05 mgm. in 2 hours and 1.7 mgm. in 4 hours.

c. In one unanesthetized dog intraperitoneal insertion of solution representing 100 mgm. of SMD per kg. resulted in blood levels of 16.8 mgm. in 15 minutes, 13.4 mgm. in 60 minutes, 11.9 mgm. in 2 hours, 10 mgm. in 4 hours, 8.4 mgm. in 5 hours and 7.5 mgm. in 6 hours. Another dog received stock solution equivalent to 200 mgm. per kg. intraperitoneally and attained blood levels of 26.4 mgm. in 15 minutes, 30.4 mgm. in 30 minutes, 25 mgm. in 60 minutes, 24.3 mgm. in 2 hours, 22.3 mgm. in 3 hours, 19.6 mgm. in 4 hours, 17.6 mgm. in 5 hours and 15 mgm. in 6 hours.

These results are convincing although sparse and if confirmation is gained by further experimentation, intraperitoneal insertion of this type of solution in appropriate amounts is suggested in generalized peritonitis at appropriate intervals to maintain a bacteriostatic concentration intraperitoneally as well as to gain early and adequate blood serum levels. Intraperitoneal injection of diluted solutions might be considered in children with inaccessible veins since previous work indicates that no residual inflammatory reaction is demonstrable macro or microscopically. (Cf. accompanying abstract.)

THE AMERICAN SOCIETY FOR EXPERIMENTAL PATHOLOGY

THIRTIETH ANNUAL MEETING

Boston, Mass., March 31, April 1, 2, 3, 4, 1942

(For possible corrections in any of the following abstracts see the next issue)

Experiments with circulatory alterations in explanted kidneys. FREDERICK M. ALLEN and WILLIAM E. YOULAND. *Depts. of Physiology and Biochemistry and of Pathology, New York Medical College.* Before the previous meeting of this Society (April 1940) the authors described: *a*, the explantation of kidneys under the skin, as originally introduced by Allen for various experimental purposes; *b*, the acute elevation of blood pressure occurring during temporary circulatory obstruction produced by clamping or ligating the pedicles of such kidneys (explained as nervous in character, a humoral renal influence being excluded); *c*, the possibility of prolonged survival of kidneys even when explanted entirely outside the skin. The present paper describes various further alterations of environment and circulation of the kidneys, and their consequences.

Chronic progressive sporotrichosis in mice. ROGER DENIO BAKER. *Dept. of Pathology, Duke Univ. School of Medicine, Durham, N. C.* It is possible to produce chronic progressive sporotrichosis in the legs of mice by single injections of suspensions of *Sporotrichum schencki* into the foot pads. Great swelling, abscess formation, and ulceration are prominent features, and the draining lymph node may become abscessed, but the health of the mouse is apparently usually unaffected, and generalization of the disease has not occurred from this mode of injection even after a period of several months.

An equivalent single injection of the killed organisms produces only minimal temporary swelling of the foot.

The condition produced experimentally in the mouse thus has much in common with sporotrichosis in the human occurring in an extremity. On the other hand, several aspects are quite different. In the mouse, lesions following the the course of lymphatic vessels are not to be

made out as in the human, the draining lymph nodes are more involved than in the human, and the tissue forms of the organism are readily demonstrable in the pus whereas they are usually not in the human.

Other data, regarding the effect of intraperitoneal injections of the fungus in mice, the effects in guinea pigs and rabbits, and comparative results of inoculation of seven strains of *Sporotrichum*, are touched on briefly.

The absorption of radioactive iron in human beings. WILLIAM M. BALFOUR. *Dept. of Pathology, School of Medicine and Dentistry, Univ. of Rochester, N. Y.* A series of patients have been given radioactive iron by mouth and the absorption calculated from the amount appearing in the erythrocytes. Pregnant women, in general, absorbed larger amounts than the other types studied. Patients who are iron deficient from chronic hemorrhage also absorbed relatively large amounts. Pernicious anemia patients took up little as did patients with secondary anemia not due to bleeding. A patient with hemachromatosis was studied in great detail and was found to absorb less than 1% of the dose. He is being studied to see if repeated bleedings will mobilize the iron in his tissues and thereby alter the course of the disease. A normal adult male was given a dose of radioactive iron and about 1% appeared in the erythrocytes in 10 days. He was then bled 500 cc. to furnish a stimulus toward hemoglobin formation and thereby to see if any radio-iron had been absorbed and not utilized. The blood concentration of radio-iron did not rise following the bleeding. Further evidence is thus furnished that the absorption of iron is regulated by the need for iron.

A method for adjusting the antigen-antibody ratio prior to the determination of pneumococcic antibody nitrogen. C. DALE BARRETT, JR. and JOHN T. TRIPP. *Michigan Dept. of Health Laby., Lansing.* The method reported provides a rapid means for adjusting the ratio of antigen to antibody prior to the determination of antibody N in antipneumococcic serums and concentrates. It is particularly applicable to the simultaneous analysis of a large number of samples of widely differing antibody content.

The agglutinative titer indicates the approximate amount of antibody present, and when properly interpreted this titer has a semi-quantitative significance. It is on the basis of this titer that an unknown pneumococcic antibody-solution is classified as to its relative antibody-concentration. When this is known, the volume of bacterial antigen required for complete precipitation of the antibody in a given aliquot can be estimated, thereby eliminating the necessity of adding the antigen in successive small increments until no antibody remains in the supernate.

The effect of a deficiency of vitamin A on ciliary motion in rat's trachea. M. A. BLANKENHORN, MAURICE LANDY and JANE BLANKENHORN. *Univ. of Cincinnati Dept. of Medicine, and the Research Labs. of the S.M.A. Corporation, Chagrin Falls, O.* This work was undertaken to learn what happens to ciliary motion in animals that are kept on deficient diets that result in replacement of ciliated epithelium by squamous non-ciliated epithelium. The technique to produce such lesions by dietary means is well-known, but there is no method of studying the ciliary motion in this site in living animals.

We have observed ciliary motion under a microscope in rings cut from freshly removed trachea, using the time such motion persists as a measure of normality. Conditions were kept as near physiologic as possible.

Twenty-four normal albino rats of various age and weight were studied. The longest duration was 12 hours and the shortest was 6 hours. The average of the 24 rats was 8.4 hours. Seven young rats of 100–120 grams weight, showing mild to severe symptoms of vitamin A deficiency presented ciliary motion of the same nature, except that scattered parts of sections showed no motion. Where motion occurred it persisted for 8.5 hours as the longest and 6.25 hours as the shortest period. The average of the seven rats was 7.5 hours.

Histologic study of these trachea showed variable degrees of metaplasia with patches of complete replacement by stratified squamous non-ciliated epithelium characteristic of vitamin A deficiency.

It is concluded that ciliary motion was not affected by diet deficient in vitamin A.

Thiamine benefits vitamin B deficient rats receiving only dextrose. J. L. BOLLMAN. *Division of Experimental Medicine, The Mayo Foundation, Univ. of Minnesota.* Young rats weighing 65 to 90 grams were maintained for 14 to 21 days on a vitamin B free diet (casein 10, sucrose 80, butter 4, salts, agar and vitamin A and D). At this time these animals survive fasting but two to three days. Substitution of a diet of dextrose, supplemented by dextrose in Ringers solution by stomach tube, permits survival of from seven to fourteen days. The survival with dextrose is somewhat less than that of the animals continued with the vitamin B free diet. The subcutaneous injection of a mixture containing thiamine hydrochloride, riboflavin, nicotinamide, pyroxidine hydrochloride, and pantothenic acid three times each week produced a rapid gain in weight of the rats receiving the vitamin B free diet and they appeared like normal rats for several months.

Rats receiving the vitamin mixture and dextrose survived two to four times longer than those receiving only dextrose. Rats receiving thiamine hydrochloride lived on dextrose as long as those receiving the entire vitamin mixture. Nicotinamide appeared to have some effect, but riboflavine, pyroxidine hydrochloride, pantothenic acid, and choline had no appreciable effect in prolonging the life of the dextrose fed rats. The addition of choline to the vitamin mixture had no further effect.

When dextrose was substituted immediately following an adequate vitamin containing diet, rats survived nineteen to thirty-five days. The injection of thiamine hydrochloride or the vitamin mixture did not appreciably alter the survival time of these animals.

The mitotic activity of a group of colchicine-like compounds. CHARLES F. BRANCH. *Dept. of Pathology, Boston Univ. School of Medicine, Boston, Mass.* It has been previously established that colchicine is a toxic drug having a marked effect on cell division. Several investigators have attempted to find a substitute or derivative of comparable activity but less toxic in character. The present study is concerned with the investigation of a group of twelve such compounds and observations of their effect on mitotic activity. For each experiment we used fifty C57 mice, planted with sarcoma 37. The compound to be tested was injected subcutaneously in varying doses and the mice were sacrificed at regular intervals up to twenty-four hours. Compounds varying considerably in their chemical structure but which are definitely less toxic, produce mitotic showers comparable to those initiated by colchicine.

Spontaneous primary hepatomas in mice of strain C3H. II. The effect of estrogens and testosterone on the incidence. EDWARD L. BURNS and JOHN R. SCHENKEN. *Dept. of Pathology and Bacteriology, Louisiana State Univ. School of Medicine, New Orleans.* In an earlier study we reported the occurrence of spontaneous primary liver cell tumors in strain C3H mice. All of these tumors occurred in mice above 12 months of age and the incidence was higher in males than in females. The present study confirmed these observations and showed, in addition, that the highest incidence of tumors occurred in breeding males. Non-breeding males, and breeding and non-breeding females showed a relatively low incidence of neoplasms.

Non-breeding male C3H mice, treated with either a-estradiol benzoate or keto-hydroxy estrin showed a higher incidence of hepatomas than non-breeding male controls of the same age, but a somewhat lower incidence than breeding male controls. Non-breeding female C3H mice treated in the same way, did not develop liver tumors. The incidence of hepatomas in non-breeding male and female C3H mice treated with testosterone proprionate did not differ significantly from that observed in non-breeding controls.

Microscopic examination showed neoplasms composed of cells closely resembling normal liver cells. In all tumors the cytoplasms of many cells contained round inclusion bodies of varying size.

Pathological changes in tryptophane deficiency. WILHELM BUSCHKE, ANTHONY A. ALBANESE and RICHARD H. FOLLIS, JR. *Dept. of Ophthalmology, Pediatrics and Pathology, Johns Hopkins Univ., Baltimore, Md.* The demonstration will illustrate the pathological lesions found in rats on a tryptophane-free diet. The most striking changes are found in growing epithelial structures. Two types of tryptophane cataract have been encountered—an acute and a chronic type. The peculiarities of these lesions as contrasted with other types of cataract will be illustrated, as well as the response to tryptophane therapy. In the cornea tryptophane deficiency induces vascularization, a lesion that will be compared with that of riboflavin deficiency. Tryptophane deficient animals develop alopecia with characteristic histological changes in the skin. The dental enamel likewise shows distinctive lesions. A variety of visceral lesions have been encountered, notable among which are those in the stomach, where metaplasia of the epithelium occurs.

Factors influencing the metabolism of radioactive iron in human subjects. MILAN A. CHAPIN and JOSEPH F. ROSS. *Evans Memorial, Mass. Memorial Hospitals, and the Dept. of Medicine, Boston Univ. School of Medicine, Boston, Mass.* Iron metabolism in human subjects has been studied with the radioactive tracer technique. Assuming that appearance of iron in circulating hemoglobin is an index of iron absorption, we observed the effect of different degrees and types of anemia, and varying states of iron reserves on iron absorption.

One milligram of labelled iron per kg. body weight was administered by mouth to each subject. Normal human adults with adequate iron reserves utilized less than 1 per cent of single doses of ingested iron. Patients with iron deficiency anemia and depleted iron reserves absorbed 3–10 per cent of similar doses. Patients with untreated pernicious anemia who presumably had adequate reserves of iron absorbed less than 1% of the ingested iron, even though the degree of anemia was extreme. The tissue reserves of iron in these patients were then decreased by repeated phlebotomies, by restricting intake of dietary iron, and by causing hemoglobin regeneration with liver extract. In this state, iron absorption was 5–10 times greater than it had been in the same patients when the anemia was much more

severe, but when iron reserves were more adequate.

Thus, the reserves of iron in the body, rather than the degree of anemia appear to determine the amounts of iron which will be absorbed.

Our observations also indicate that the amount of labelled iron utilized for hemoglobin synthesis is independent of the valence state in which the iron is ingested.

Human neoplasms in tissue culture. DALE REX COMAN. *McManes Laby. of Pathology, Univ. of Pennsylvania School of Medicine, Philadelphia.* Observations upon living neoplastic cells have been confined largely to those obtained from lower animals. No adequate systematic study of the behavior of human living neoplastic cells has been made. It was believed that such a study might increase our knowledge of the requirements for continued growth of human neoplastic cells, the capacity for movement and the growth potential of cells derived from different tumors and a host of related problems. The object of this first study of the contemplated series was to find a satisfactory method of maintaining human neoplastic cells in vitro and to observe the general growth characteristics of benign and malignant living neoplasms. Since studies of human tumors have usually depended upon their microscopic appearance in fixed and stained preparations, a comparison between the microscopic appearance of the original tissue and its appearance while growing in vitro was made.

Human neoplastic tissues were cultured by the roller tube method. The neoplasms studied included fibro-adenomas, adeno-carcinomas, squamous cell carcinomas, an hemangiosarcoma, mixed tumors of the parotid gland, lymphosarcomas and others.

Excellent growth was obtained from these tumors. In instances the cultured tissues reduplicated quite closely the structure of the original tumor, whereas in others, differences developed. Neoplasms closely resembling each other in the original, in tissue cultures exhibited strikingly different behavior. These variations were noted both in the neoplastic cells and in the stroma. The behavior of the living cells could not be foretold from an examination of histological sections of the original tissue.

Some ultracentrifugal properties of complement. HARRY EAGLE and MORRIS ROSENFELD. *U. S. P. H. S., Washington, D. C., and the Depts. of Medicine and Pharmacology, Johns Hopkins Medical School, Baltimore, Md.* The ultracentrifugation of fresh guinea pig serum, either whole or diluted, for three to six hours at 60,000 r.p.m., causes the complete destruction of its hemolytic (complement) activity. This is due primarily to the inactivation of the so-called midpiece fraction of the complement, and to a lesser extent, of the so-called endpiece fraction. This destruction of complement activity is not observed if NaCl is added to the serum to a concentration of 8.5 per cent. In such whole salted serum, the midpiece and endpiece fractions of complement sediment at differing rates. The apparent sedimentation constant of endpiece in whole salted serum (2.3-2.6 S) is approximately that of the serum proteins under the same conditions; while the regularly higher sedimentation constant of the midpiece (3.1–2.4 S) suggests a larger molecule.

The relation of antibodies in human beings to immunity and infection with influenza A. MONROE D. EATON and WALTER P. MARTIN. *Research Laby., California State Dept. of Public Health, Berkeley.* Studies by complement fixation and mouse neutralization tests on persons vaccinated or infected with the virus of influenza A revealed that the resultant degree of increase in antibodies was inversely related to the initial titer. In a group with low antibody levels before vaccination or infection, the proportion of persons showing significant increases was greater than in a group with high antibodies. The results suggest that infection with influenza A virus is sometimes not detectable by serological tests when the antibody titer is high at onset. During an epidemic caused predominantly by the virus of influenza A, cases of clinical influenza occurred with about equal frequency in persons with low, medium, or high titers of serum antibodies. In a group of individuals that had been vaccinated and subsequently contracted clinical influenza, the serum antibodies at onset were higher than in the comparable non-vaccinated group, but the percentage of cases positive for influenza A was lower than among the non-vaccinated cases. The results indicate that the higher antibody levels in vaccinated persons may interfere with the serological diagnosis of influenza A, thus leading to errors in the interpretation of the efficacy of vaccines.

Among a total of 1,102 persons vaccinated the incidence of clinical influenza was 8.3 per cent, and the incidence of influenza A (as judged by specific increase in antibodies) was 4.2 per cent. Among 7,987 controls the incidence of clinical influenza was 12.3 per cent and of influenza A 9.3 per cent.

Effects of pregnancy on blood pressure in chronic hypertensive and normal dogs. CYRUS C. ERICKSON. *Dept. of Pathology, Duke Univ. Medical School, Durham, N. C.* These experiments show that chronic hypertensive dogs characteristically demonstrate a depression of blood pressure during the terminal period of pregnancy; and that normal dogs, similarly, may show a tendency to a comparable though less marked decline in blood pressure levels in the same period of pregnancy. This

lowered level of the blood pressure persists for varying periods following delivery.

In our experiments we have utilized the methods of Goldblatt and coworkers for producing the experimental hypertension; and the blood pressures have been determined by the direct femoral artery puncture method. Three groups of animals are described: *a*, chronic hypertensive dogs observed for as long as four years during repeated pregnancies; *b*, chronic hypertensive dogs each studied during a single pregnancy, and *c*, normal dogs observed during a single or multiple pregnancies.

These experiments observing the blood pressure during pregnancy in dogs with pre-existing hypertension are compared with our previously reported experiments in which hypertension was induced in dogs during the last trimester of pregnancy.

These observations suggest that the altered physiological balance accompanying pregnancy in both hypertensive and normal animals may be quite similar. Previously reported observations record the fact that depressions of blood pressure in normal and hypertensive pregnant animals may be accentuated during periods of hypoproteinemia induced by the administration of gum acacia or by plasmapheresis.

Effect of repeated doses of radiation of mouse sarcoma 180 on abnormalities of mitosis. LLOYD C. FOGG and SHIELDS WARREN. *Laby. of Pathology, New England Deaconess Hospital, Boston, Mass.* The quantitative studies reveal an increase in the per cent of multicentriolar cells and a decrease in the abnormal mitosis to the usual frequency. Increase in the number of cells capable of several abortive divisions is indicated.

Vitamin K anemia in experimental animals. O. M. GRUHZIT. *Research Labs., Parke, Davis and Company, Detroit, Mich.* Toxicologic studies of vitamin K substances in animals revealed the development of hemolytic anemia. Oral administration of 15 mgm./kgm. of 1,4-naphthoquinone or of similar doses of its derivatives invariably caused R.B.C. destruction in dogs. 2-Methyl-1,4-naphthoquinone in two weeks produced R.B.C. drop of 1.67 million per c.mm. 4-Amino-2-methyl-l-naphthol reduced the R.B.C. by 0.87 million in two weeks and in three weeks the count decreased to 2.51 million per c.mm.; the hemoglobin values dropped to 43 Sahli. From the fourth week the animals established a tolerance and the blood cytology tended to return to normal levels in spite of continued administration. Intravenous administration of vitamin K was followed by more constant and progressive R.B.C. destruction. The W.B.C. at first increased and then gradually dropped to 2,500 per c.mm. In the most profound cases of anemia, the reticulocytes did not rise above 4.7 per cent. The blood platelets became depressed in the initial stages, but surpassed the normal count at the height of the R.B.C. destruction. The low reticulocyte response and the development of leucopenia suggest that vitamin K substances, under conditions of prolonged administration of doses 20 to 150 times larger than those used clinically, may produce a hypoplastic type of anemia similar to the anemia due to benzol derivatives and not like that due to disulfides.

Circulating red cell mass as a linear function of the venous hematocrit in the dog. P. F. HAHN. *Dept. of Pathology, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.* Radioactive iron when fed or administered by vein to an anemic, iron depleted dog is quite promptly utilized, and the circulating red blood cells are then tagged. Such an animal may be used as a donor in the study of the total and circulating mass of red cells in other dogs. If a known volume of such donor cells representing a measured amount of radioactivity are then injected into a recipient, the subsequent dilution of the tagged cells allows a direct determination of the volume of red cells in circulation. After complete circulation of the blood in the body another sampling allows an estimate of the total red cell mass.

The red cell hematocrit may be varied by controlled hemorrhage and repeated determinations of the circulating mass of cells at various hematocrit levels may be made in the same dog. As can be predicted by other means it is found that within the limits of error of the experimental method used, the circulating mass of cells is a linear function of the venous hematocrit. Such a relationship permits one to estimate the red cell volume at any hematocrit level in a given dog provided the linear function has been previously determined for this animal. Its use in the study of isotopes, blood volume and shock procedures will be briefly pointed out.

Experimental allergic inflammation of the intestine. GEORGE HARTLEY, JR. *Evans Memorial, Mass. Memorial Hospitals, and the Dept. of Pathology, Boston Univ. School of Medicine, Boston, Mass.* Previous work suggested that ingested antigen is localized in the intestinal wall by specific antibodies. Because the work of Opie and others has shown that allergic inflammation in the skin (the Arthus phenomenon) is caused by a localization of antigen by antibodies, the demonstration of such a reaction in the intestine would prove that antigen is localized by antibodies in this organ.

Following the injection of crystalline egg-albumin into the tied-off cecum or directly into the wall of the cecum of hypersensitive rabbits, a severe inflammatory reaction developed locally. A similar inflammatory response was produced in the ileum of hypersensitive rabbits following the

injection of antigen into a small radical of the mesenteric artery. The inflammation was characterized by a marked neutrophilic infiltration of the entire wall (mucosa, sub-mucosa, muscular layers, and serosa), edema and slight hemorrhage of the sub-mucosa, and blood vascular congestion of the sub-mucosa and sub-serosa. At the site of the mesenteric arterial injections, an acute arteritis and periarteritis developed. No lesions were present in the control normal rabbits that were treated in the same manner as the hypersensitive animals. All of the above lesions were present after twenty-four hours.

These experiments demonstrate the direct production of allergic ileitis for the first time. Since it has been definitely established that allergic inflammation in the skin is the result of the localization of antigen by antibodies, these experiments prove that antigen is localized by specific antibodies in the intestinal wall.

The isolation of a polysaccharide from amyloid-bearing tissues. GEORGE M. HASS. *Dept. of Pathology, Cornell Medical College, New York City.* A polysaccharide was isolated by semi-quantitative methods from amyloid-bearing spleens and livers obtained postmortem from patients with chronic pulmonary tuberculosis. Control hepatic and splenic tissue from similar sources but without microscopic deposits of amyloid did not contain significant amounts of the polysaccharide. The compound was recovered only under conditions which led to solution of the amyloid matrix. The amount which was isolated was directly proportional to the estimated quantity of amyloid. The yields indicated that no more than 1.5 per cent of the amyloid matrix was composed of the polysaccharide. The properties and principal degradation products of the preparations were quantitatively and qualitatively similar, not only to one another, but also to samples of chondroitin sulfuric acid obtained from infantile cartilage.

Protein-antiprotein reactions in horse sera. MICHAEL HEIDELBERGER, HENRY P. TREFFERS and JULES FREUND. *Dept. of Medicine, College of Physicians and Surgeons, Columbia Univ. and the Presbyterian Hospital, New York, and the Research and Antitoxin Laby., New York City Department of Health, Otisville, N. Y.* The behavior of antibody in horse sera is of two types. In one the curve passes through the origin, i.e., precipitates are formed with relatively small additions of antigen. In the other there is a definite prezone and no precipitate forms until a relatively large amount of antigen is added. Only anticarbohydrate systems have been reported as of the first type, while the second has been thought to be typical of antiproteic, including antitoxic, systems in horse sera. It is now shown that both types of curves may be obtained with antiprotein in horse sera.

Two horses were injected with rabbit serum proteins. Quantitative precipitative analyses of the serum from a horse which received intravenous injections of alum-precipitated rabbit globulin showed curves typical of those obtained with antiprotein in rabbit sera and with anticarbohydrate in horse sera. Similar behavior was observed for pneumococcal protein used as antigen with an antipneumococcal horse serum of high antiprotein content. On the other hand, serum from a horse which received subcutaneous injections of alum-precipitated rabbit albumin gave curves resembling other antiproteic systems previously reported.

Fixation of complement nitrogen and complement components by specific precipitates. MICHAEL HEIDELBERGER, OTTO G. BIER (Sao Paulo) and MANFRED MAYER. *Depts. of Medicine and Biochemistry, College of Physicians and Surgeons, Columbia Univ. and the Presbyterian Hospital, New York City.* Paralleling an as yet unpublished study by Ecker and Pillemer of titer reductions of the components (C′1, C′2, C′3, C′4) of complement (C′) in antigen-antibody combination a correlation was made between nitrogen uptake by suitable specific precipitates and the reduction in titer of the components of whole C′ and of C′ deprived of one or more components by standard procedures C′ N in whole complement as determined by addition to specific precipitate N under the conditions used is derived from practically all of the C′1 and C′4. Titer reductions of C′2 and C′3 are less complete and the contribution of these components to C′N is uncertain. Analytical data are presented and an attempt is made at partial elucidation of the complex relationships observed with the various groups of C′ components in heated, ammonia-treated, CO_2-fractionated, and zymin-treated C′.

On a series of 9 human fertilized ova (5 normal and 4 pathological) obtained in surgically removed uteri prior to the first missed menstrual period. ARTHUR T. HERTIG and JOHN ROCK. *Free Hospital for Women, Brookline, and the Depts. of Pathology, Gynecology, and Obstetrics, Harvard Medical School, Boston, Mass.* This series of pathological fertilized human ova was obtained during the course of a broader study on human development occurring prior to the first missed menstrual period. Fifty married women, 20 to 40 years of age, scheduled to have a therapeutic hysterectomy, were selected from the regular waiting list of this hospital. Menstrual and coital data were recorded in the interval preceding operation which was scheduled from the 15 to the 33rd day of the menstrual cycle. Specimens were opened laterally under the binocular dissect-

ing microscope and fixed in Buin's fluid within 40 minutes.

Nine uteri contained early implanted ova; five normal and four pathological. The patients' average age was 32.3 years, (35.3 years for non-pregnant group) with no significant difference between the ages of the normal and abnormal group. Their obstetrical histories revealed that the five patients yielding normal ova had had 31 full term normal pregnancies, 1 stillbirth, one premature infant, and 3 spontaneous abortions, whereas the four yielding abnormal ova had had 22 full term normal pregnancies, 3 stillbirths and 6 abortions.

The defects in the pathological ova varied from absence of embryonic rudiment (the most common finding in spontaneously aborted ova) to localized defects in the trophoblastic development. Since the endometria are free from any gross or microscopic stigmata of disease, and since all of these patients had each produced at least 4 healthy full term infants, it would appear that intrinsic defects in the fertilized zygote would best explain the future probable abortion.

Experimental arterial lesions related to diet and renal insuciffiency. RUSSELL L. HOLMAN. *Dept. of Pathology, School of Medicine, Univ. of North Carolina, Chapel Hill.* Acute necrotizing arterial lesions affecting principally the large elastic arteries (aorta, endocardium of the left auricle, coronary, pulmonary and other arteries) appeared unexpectedly during the course of experiments designed to determine whether heavy metal poisoning is influenced by altering the plasma protein level. Five dogs maintained on a standard diet were made hyperproteinemic by repeated intravenous injections of plasma obtained from healthy donor dogs. Each then received a single subcutaneous injection of uranium nitrate (5.0 mgm./kgm.). Acute necrotizing arterial lesions were found in the 4 dogs that died 8–17 days after administration of the heavy metal; and the 5th dog showed healed lesions in the pulmonary aorta when it was sacrificed 11 months later. Control data indicated that the lesions were related to the experimental procedure and were not due to accidental or coincidental infection.

Since reporting these early experiments 2 dogs subjected to the same experimental procedure except that mercuric chloride (3 mgm./kgm. *intravenously*) was used instead of uranium nitrate, and 3 dogs subjected to bilateral nephrectomy instead of heavy metal poisoning have all shown similar lesions. These experiments seem to indicate that renal insufficiency is the essential factor.

Evidence for a dietary factor is incomplete but consists of 4 dogs subjected to the same experimental procedure except that they were fed the regular kennel diet of meat scraps and bones instead of the standard diet. None of these showed arterial lesions.

Effect of vasoactive preparations on renal circulation of exteriorized kidneys. BENJAMIN JABLONS. *Polyclinic, Welfare and City Hospitals, New York City.* Exteriorization of the kidneys in animals was the logical development of the early experiments of Minkowski, Allen, Barcroft et al. to explant visceral organs under the skin. These experiments demonstrated that change of position of these organs did not interfere with their viability or function. Studies of Allen, and the author have shown that it is possible not only to explant the kidney subcutaneously but even to exteriorize this organ successfully in dogs, rabbits and rats. Animals with one exteriorized kidney have survived for periods as long as four months. This type of preparation makes possible the study of the effect of various procedures on the renal circulation and also the effect of hypertensive and hypotensive agents on the kidney as reflected in surface temperature changes. In addition, it is possible to influence blood pressure levels by certain procedures. Exteriorization of the kidneys is followed by histological changes in the capsule and in the renal parenchyma.

The reactivation of neutralized phage and other biologic agents by digestion of the antibody with papain. G. M. KALMANSON and J. BRONFENBRENNER. *Dept. of Bacteriology and Immunology, Washington Univ. School of Medicine, St. Louis, Mo.* In the preliminary experiments it was found that mixtures of phage and antiserum which are just neutral can be reactivated by digestion of antibody by papain, whereas overneutral mixtures failed to be reactivated under similar conditions.

The failure of reactivation of overneutralized mixtures could have been due either 1, to the prolonged time of contact between phage and antibody, or 2, to the fact that as neutralization proceeded each phage particle took up more antibody than the minimum needed to neutralize it. In order to test these alternative possibilities, "equilibrium" mixtures of phage and antiphage were made in which there was just enough antibody to neutralize the amount of phage present, whatever the period of contact, and "overneutral mixtures" in which the same amount of serum was allowed to combine with phage reduced one-hundredfold. It was found that the "just neutral" mixtures could be reactivated even after a prolonged contact (56 days) while the overneutral mixtures were unreactivable at any time.

Experiments in progress indicate that the failure of reactivability of the overneutralized mixture may be due to the failure of the papain to completely destroy the antibody containing molecule (as opposed to its antibody function).

Similar experiments were carried out with the

pneumococcus-antipneumococcus, and botulinus toxin-antitoxin systems. The latter gave results similar to those obtained with phage, while the pneumococcus, even when neutralized to the extent of complete loss of infectivity due to a gross excess of antibody, was rendered infectious to about the original degree upon digestion with papain.

The serum concentration and renal clearance of potassium in severe renal insufficiency. NORMAN M. KEITH, HARRY E. KING and ARNOLD E. OSTERBERG. *Mayo Clinic, Rochester, Minn.* A normal individual daily ingests and subsequently excretes in the urine several grams of potassium. This fact suggests the possibility of renal retention of potassium in the blood when the kidneys are seriously damaged. Therefore, chemical studies of the blood, including the concentration of potassium in serum, were made in thirty-three patients with renal insufficiency; in eight of these the renal clearance of potassium and urea were also estimated. Subsequently, in fourteen of the patients necropsy was carried out and histopathologic study of the kidneys made. We confirmed the apparent paradoxical finding of previous investigators, that the concentration of serum potassium in renal disease may be normal, decreased or increased. Among extrarenal factors affecting the serum potassium level are the food intake, vomiting, diarrhea and the rate of metabolism. The presence of an increased concentration, 30 mg. per cent or more, in serum indicates a serious prognosis and occurs when total renal function is markedly reduced, as for example, in rapidly progressive acute, subacute and chronic glomerulonephritis, severe long-standing passive congestion and terminal renal states. An interesting fact is that in patients, who have chronic uremia, the fasting renal clearance of potassium may be normal when the simultaneous clearance of urea may be reduced to 3 cc. per minute. This finding indicates that even when the renal parenchyma is almost totally destroyed, it may retain the function to concentrate potassium in the urine to above the concentration in the serum and thus the ability to excrete the daily basal amount of potassium.

The Rh factor in the pathogenesis of erythroblastosis fetalis. PHILIP LEVINE. *Division of Laboratories of the Newark Beth Israel Hospital.* Recent studies show that the Rh factor of fetal blood was responsible for isoimmunization of 90 per cent of more than 300 mothers of erythroblastotic infants. In this group of Rh negative mothers, the husbands and the affected infants were invariably Rh positive. The source of the intrauterine hemolysis of fetal blood is the anti-Rh agglutinin which could be demonstrated in about 40 per cent of the mothers if tested shortly after delivery of an affected infant.

In the remaining 10 per cent, i.e., Rh positive mothers, other blood factors may induce the isoimmunization. One of these is identified as Hr which is genetically related to Rh (Levine and Javert).

The familial nature of erythroblastosis depends upon the heredity of the Rh factor shown by Landsteiner and Wiener to be a dominant. The striking familial incidence in certain mothers or its sporadic occurrence in others depends upon the homozygosity or heterozygosity of the father's blood.

A complicating feature is the variety of anti-Rh agglutinins. Anti-Rh_1 sera give either 13 or 15 per cent negative reactions, while anti-Rh_2 sera give about 30 per cent negatives.

These studies paved the way toward the prevention of transfusion accidents attributable to isoimmunization by the Rh factor in the fetal blood.

Isoimmunization is also the cause of many stillbirths and miscarriages. Studies of the Rh factor have been initiated to determine the role of isoimmunization in the following conditions: toxemia of pregnancy, premature separation of placenta, anemia of pregnancy, malformation, and spastic paralysis (kernicterus).

The changes in blood count associated with radiations from the cyclotron. HERMAN LISCO and SHIELDS WARREN. *Laby. of Pathology, New England Deaconess Hospital, Boston, Mass.* Those individuals working with cyclotrons are subjected to minute amounts of radiations whose biologic effects have not yet been evaluated. These workers exposed to the varying radiations and studied here number 78. For over two years, the blood counts and the morphology of the blood cells of the workers on the Harvard cyclotron and the Massachusetts Institute of Technology cyclotron have been followed. In general, so long as the workers are adequately protected, no change in the bloods has been noted. Certain workers gradually developed leukocytosis with relative lymphocytosis. Their hematopoietic apparatus has been considered too unstable to allow them to continue with safety the work about the cyclotron. No permanent ill-effects have been noted, although temporary anemia may develop.

A comparison of the collodion particle technique with other methods of measuring antibody. FRANCIS C. LOWELL. *Evans Memorial, Mass. Memorial Hospitals, and the Dept. of Medicine, Boston Univ. School of Medicine, Boston, Mass.* Rabbit antisera for a number of protein antigens were prepared and tested for antibody content by the collodion particle technique, the quantitative antibody dilution complement fixation test, the gelatin

antibody dilution interface test (Hanks, 1935) and the antigen dilution interface (ring) test. When possible the antibody nitrogen content of the sera was determined. It was found that the collodion particle technique was the most sensitive of the methods used and gave a reliable indication of the strength of the antiserum. For example, an antiovalbumin serum containing 0.79 mgm. antibody nitrogen/cc., gave definite agglutination of ovalbumin-coated collodion particles in dilutions of 1:8192 and 1:16,284. The same serum failed to give a visible ring with the gelatin antibody dilution interface test when diluted more than 1:1024. Complement fixation failed to occur when the serum was diluted more than 1:200 or 1:400. A titer of 1:57,000 was obtained with the antigen dilution interface test with 4 per cent ovalbumin solution. The antigen dilution interface test, however, did not give a reliable indication of the strength of the antiserum.

The application of the collodion particle technique was limited to certain protein antigens. The sensitivity and reliability of the method appeared to depend on the thorough and permanent coating of the particles with the antigen.

It is concluded that, of the methods used, the collodion particle technique is the most sensitive and that it is a reliable method for measuring the antibody content of rabbit antisera.

The effects of temperature on growth and metastasis of spontaneous frog carcinoma. B. LUCKÉ and H. SCHLUMBERGER. *Laby. of Pathology, School of Medicine, Univ. of Pennsylvania, Philadelphia.* Two groups of frogs having large spontaneous renal carcinomas were maintained in thermostat rooms at 7°C and 28°C respectively. A third, control group, was kept at room temperature. None of the animals were fed. Periodic examinations of the tumors were made by x-ray photographs after an intracoelomic injection of air. Upon death of the animals the post mortem findings were compared with the x-ray films.

Of the frogs maintained at a low temperature 21 survived for from 58 to 109 days; at the high temperature 27 survived for from 21 to 84 days. In the control group of 26 frogs the survival period ranged from 20 to 220 days. No significant changes in the size of the spontaneous tumors could be attributed to the effects of temperature. Temperature did, however, profoundly influence the incidence of metastasis. Whereas in the group kept at a low temperature only two of the frogs, i.e., 9 per cent, developed metastases, and these, single nodules of small size; at the high temperature over 50 per cent of the animals were found to have metastases, usually multiple and large. In the control group metastasis occurred in only a single animal, an incidence similar to that found in the group kept at low temperature.

These results may be correlated with the effect of temperature on the systemic circulation and on the motility of the tumor cell.

The effect of the intravenous injection of alkaline intestinal phosphatase on dogs following hepatectomy and total abdominal visceration with and without hepatectomy. STEPHEN MADDOCK, GERHARDT SCHMIDT and SIGFRIED J. THANNHAUSER. *Boston City Hospital and the Boston Dispensary.* In a previous communication one of us described the effects of hepatectomy and evisceration on the alkaline serum phosphatase of the dog. It was found that hepatectomized animals showed a marked rise in phosphatase in the post-operative period. Abdominal visceration without hepatectomy produced no rise and abdominal evisceration with hepatectomy gave a slight rise. These results were interpreted as indicating that the liver acted as a regulator of phosphatase in the serum.

The discovery of a method for making a highly purified phosphatase solution made it possible to test this theory from another angle. Normal and hepatectomized dogs were given a sufficient intravenous dose of phosphatase to raise the serum level to the neighborhood of 400 Bodansky units per 100 cc. The normal dog showed a sharp fall to the neighborhood of 100 units or less in 5 hours and then a gradual fall to normal. The hepatectomized animals showed a progressive rise to the neighborhood of 1000 units in 10–11 hours.

Animals with abdominal evisceration without hepatectomy fell more rapidly than normal dogs. Nephrectomized animals behaved essentially like the normal. When the liver remains intact there is a disappearance of intravenously injected phosphatase even following a shocking operation such as abdominal evisceration. In hepatectomized animals there is always a great increase in the serum phosphatase. These results would seem definitely to bear out the theory expressed above.

The results of in vitro experiments will be discussed.

Use of the chick embryo for evaluation of the efficacy of drugs in tuberculosis. C. M. MCKEE and E. ALTURE-WERBER. *Division of Microbiology of the Squibb Inst. for Medical Research, New Brunswick, N. J.* Goodpasture and Anderson, Costil and Bloch, Moore and Emmart have reported studies on infection of the chorio allantoic membrane with tubercle bacilli. The last mentioned author suggests this as a method for studies in chemotherapy.

In the work reported here infection of the egg was achieved by yolk sac inoculation using both human and bovine strains of tubercle bacilli. The human strain was used in most of the chemotherapeutic studies.

A light suspension of tubercle bacilli was in-

jected into the yolk sac of six day eggs. The following day the drugs, in suitable dilution, were injected into the yolk sac or into the allantoic cavity. Subsequentin jections of the drugs were sometimes made at two to three day intervals. Eggs were opened for examination 12 to 14 days after infection. At this time the chorio allantoic membranes of untreated eggs showed many discrete tubercles and, in stained films of the yolk sac, many clumps of tubercle bacilli could be seen. The tissues of the embryo were also invaded by the bacilli. For comparative purposes, however, the degree of infection has been judged on the basis of the number of tubercles in the allantoic membrane and the number of stained bacilli in films of the yolk sac.

Sulfathiazole and sulfdiazine both show definite inhibitory action in about equal degree. Sulfanilamide, sulfapyridine and promin show little or no effect. These results are surprising when compared with results in guinea pigs and mice. Promin and sulfanilamide have been shown to give some protection to the guinea pig. None of the drugs have protected mice. Because of this lack of correlation further work must be done before the results obtained with any one technique can be applied to the therapy of tuberculosis in man.

The passage of radioactive iron through the human placenta. W. T. POMMERENKE. *Dept. of Obstetrics and Gynecology, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.* The fetal requirement of iron is about 400 mgms. The exact manner of passage of this element through the placenta is not entirely understood. Employing the radioactive isotope of iron the authors were able to recover the labelled iron which had been administered orally to the mother, within less than an hour, in the fetal circulation. Data dealing with the deposition of this iron in various fetal tissues are presented. The speed with which the transfer of iron from the mother to the fetus is effected suggests that plasma is probably the transport vehicle.

Chemotherapy and persistent infection in lymphogranuloma venereum. GEOFFREY RAKE and HELEN JONES. *Division of Microbiology, The Squibb Inst. for Medical Research, New Brunswick, N. J.* It has been shown earlier that while the sulfonamide drugs prevent death in mice inoculated with large doses of the agent of lymphogranuloma venereum, these drugs are not always curative and that the agent may continue to be carried within the tissues for long periods. The present report deals with recent careful studies which have determined the relative efficacy of the various drugs *a*, in preventing death or crippling lesions from acute infection and *b*, in sterilizing the animal body to prevent the establishment of the carrier state. In addition studies have shown that changes in virulence and in susceptibility to sulfonamide drugs may occur in the strains of the agent isolated from carrier mice. No evidence of transmission of these strains from carriers even to intimate contacts has yet been obtained.

Poliomyelitis produced directly in monkeys with virus derived from "neurotropic" streptococci. E. C. ROSENOW. *Division of Experimental Bacteriology, Mayo Foundation, Rochester, Minn.* The strain of streptococcus from which the virus was derived was isolated from the nasopharynx of a patient in the acute stage of poliomyelitis. The presence of natural virus was excluded by making serial dilution cultures representing dilution of at least 10^{-20}, and by a series of subcultures from single colonies. The strain was then grown in autoclaved chick-mash medium, cultures and filtrates of which yielded virus "takes" directly in monkeys.

The symptoms and lesions in the first and second passages were chiefly encephalitic, whereas in the subsequent passages the symptoms and lesions were typical of poliomyelitis. Monkeys that had recovered from "natural" virus were resistant to this experimental virus strain.

Neurotropic streptococci were isolated from the brain and spinal cord with the same difficulty as from "natural" virus. Both emulsions and filtrates of this experimental virus produced typical symptoms and lesions. The results of this study differ from those obtained heretofore in that virulence was sufficiently high to produce fatal attacks of poliomyelitis in Macacus rhesus monkeys without passage first through mice or other animals.

On a method of decreasing the reactivity to tuberculin by the inhalation of fumes from a boiling suspension of tubercle bacilli. SOL ROY ROSENTHAL. *Tice Labys. Research Division of the Municipal Tuberculosis Sanitarium and the Dept. of Pathology and Bacteriology, Univ. of Illinois, School of Medicine.* Certain individuals exposed to the fumes of boiling tubercle bacilli were noted to develop symptoms suggestive of a generalized tuberculin reaction. After repeated exposure, these reactions abated or disappeared. It was later discovered that the degree of response to the fumes was directly related to the tuberculin reaction of the individual. Applied to animals the following observations were made: The cutaneous response to tuberculin could be appreciably reduced in guinea pigs, made reactive by the injection of avirulent tubercle bacilli, following daily exposures to: 1, fumes of boiling tubercle bacilli; 2, sprays of heat killed bacilli, and 3, fumes of boiling tubercle bacilli passed through a fritted glass filter, in the order mentioned. Vapors of old tuberculin or saline did not affect the reactivity to tuberculin. Retardation of the

development of the latter could also be accomplished by some of the above methods.

The direct determination of erythrocyte volume in human subjects with radioactively tagged cells. JOSEPH F. ROSS and MILAN A. CHAPIN. *Evans Memorial, Mass. Memorial Hospitals, and the Dept. of Medicine, Boston Univ. School of Medicine, Boston, Mass.* Determinations of plasma, cell, and blood volumes of normal human subjects have been made with the dye T-1824 and with radioactive iron tagged erythrocytes. Comparison of the values obtained by the two methods indicates that the plasma volume as usually determined is falsely low. This error is accounted for by the fact that mixing of the dye with the plasma in the vascular system is incomplete in the time usually allowed. Cell volumes calculated from the venous hematocrit and the dye plasma volume determined after *complete* mixing of the dye, are not significantly higher than those determined directly with radioactively tagged cells.

These results are quite different from those previously obtained by similar techniques in dogs. In these animals direct determinations of cell volume with radioactively tagged cells gave values for the cell volume 15–25 per cent lower than those based on plasma volume-venous hematocrit determinations. Our findings indicate that in man when time is allowed for complete mixing of plasma and dye, cell volume determinations based on the dye plasma volume and venous hematocrit may be more valid than had been believed previously.

Complement fixing antibodies (Brown-Pearce carcinoma) in the blood serum and in the aqueous fluid of the anterior chamber of the eye. O. SAPHIR, M. APPEL and M. JANOTA. *Depts. of Pathology and Bacteriology of the Michael Reese Hospital, Chicago.* It has recently been shown that immunization of rabbits to the Brown-Pearce carcinoma does not protect against growth of this tumor in the anterior chamber of the eye, even though all other tissues tested are tumor resistant.

The following study was intended to investigate the relative content of the complement fixing antibodies in the blood serum and in the aqueous fluid of the anterior chamber of the eye. The complement fixation tests were done according to the method described by Kidd, using fresh Brown-Pearce tumor tissue in the preparation of the antigen. Twenty-four tumor bearing rabbits, whose blood sera gave three to four plus complement fixation reactions, were used for this experiment. The complement fixation tests on the aqueous humor in these animals were consistently negative. It is suggested that the successful growth of the Brown-Pearce carcinoma, in the anterior chamber of the eye of the tumor immune rabbit, is due to the failure of antibodies to pass from the blood to the aqueous fluid.

It is interesting that in the re-formed aqueous humor complement fixing antibodies were present. This is in accordance with the known clinical observations that bacterial antibodies appear in the anterior chamber of the eye following anterior chamber puncture.

Some immunochemical aspects of the fibroma-myxoma transformation. HENRY W. SCHERP, GEORGE P. BERRY and DOROTHY R. SHAW. *Dept. of Bacteriology, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.* Previous investigation showed that the factors which induce the transformation of fibroma to myxoma virus are an integral part of the elementary bodies of myxoma. An immunochemical analysis of the latter was therefore made as part of an investigation of the mechanism of the transformation.

The best preparations of myxoma elementary bodies consisted of equal parts of protein containing 13.8 per cent nitrogen and 0.4 per cent phosphorus, and fat containing 1.3 per cent nitrogen and 2.4 per cent phosphorus. The $M.I.D._{.50}$ contained 6.3×10^{-13} gm. of protein, which was estimated to be equivalent in mass to 400 elementary bodies.

These preparations failed to fix complement in the presence of convalescent myxoma serum, indicating the absence of the "soluble antigens" of myxoma. Heat-inactivated preparations, which represented the "transforming factors," engendered abundant amounts of neutralizing antibodies in rabbits, but only a low level of active immunity. In only one case did antibodies to the soluble antigens of myxoma appear, following prolonged immunization.

These data supply additional evidence that the configurations of the constituents of the heated myxoma elementary body remain sufficiently intact to provide a "template" for the formation of active myxoma virus, in the presence of a developing fibromatous lesion. A large excess of "transforming factors" is, however, necessary to initiate the reaction with regularity, but in these circumstances, as little as one $M.I.D._{.50}$ of active myxoma virus will then out-multiply as many as 10,000 $M.I.D._{.50}$ of fibroma virus.

Intraocular transplantation of frog carcinoma through 15 serial generations. H. SCHLUMBERGER and B. LUCKÉ. *Laby. of Pathology, School of Medicine, Univ. of Pennsylvania, Philadelphia.* Transplants of renal carcinoma of leopard frogs have been successfully maintained in the anterior chamber of the eye through 15 serial generations over a period of two and a half years. It has thus been possible to study the characteristics of the growth of the tumor in successive generations by direct observation through the slit-lamp micro-

scope. With repeated transfer the growth of the tumor has become more vigorous and invasive. The general pattern, however, has remained remarkably stable, the form assumed being modified by the structures with which it comes in contact.

In an additional series of experiments the mode of intraocular growth of a tumor originally obtained from a metastatic nodule in the liver has been studied through six serial generations. Both in vigor and pattern these transplants were found to correspond closely to transplants of primary tumors. These results indicate that primary tumors and their metastases will grow in like manner when placed in a similar environment.

Further purification of thrombin: solubility curve of purified products. WALTER H. SEEGERS. *Dept. of Pathology, State Univ. of Iowa.* Further purification of thrombin can be achieved by simple extension of techniques previously described. The precipitate obtained at pH 5.0 (cf. J. Biol. Chem. **136:** 103, 1940, p. 104, second line from bottom of page) carries down thrombin by adsorption. This thrombin can be eluted with 10–15 cc. water kept at pH 5.0. The precipitate can repeatedly be redispersed in the mother liquors for the adsorption of more thrombin which can again be eluted with water. The activity in 19 selected eluates was found to be 11,592 ± 166 units per mgm. N. One set of test reagents was used, and additional allowance for variation must be made, because there is practically unavoidable variation in the reactivity of purified fibrinogen preparations. There is some but variable loss of activity with drying in the frozen state.

Dried material was used for obtaining solubility curves. The curve predicts that pure thrombin will possess approximatey 12,000 units per mgm. N. This figure is reliable as to order of magnitude, however, its accuracy will need confirmation. These dried preparations contain two active components with distinctly different solubility. The solvent used consisted of 5 parts Na_2SO_4 saturated at 31°, and 4 parts M/15 phosphate buffer of pH 7.2. [*Aided by a grant from the John and Mary R. Markle Foundation. Assistance was supplied by the Graduate College, State University of Iowa; certain materials and equipment were supplied by Parke, Davis & Company, Detroit.*]

Results following inoculation of egg-passaged measles virus into children by various routes. MORRIS F. SHAFFER, GEOFFREY RAKE, JOSEPH STOKES, JR., and ELIZABETH P. MARIS. *Division of Micribiology, The Squibb Inst. for Medical Research, New Brunswick, N. J., and The Children's Hospital, Philadelphia, Pa.* In attempts to reproduce certain infections, experimental procedures sometimes may not sufficiently simulate natural conditions. Thus Francis has shown that nasal instillation of fluid suspensions containing influenza A virus into presumably susceptible human volunteers seldom results in clinical grippe. Recent observations on inoculation of children by various routes with egg-passaged measles virus bear on this problem. For example in a group of 6 children in whom the virus (propagated in the chorio-allantois of the developing chicken embryo) was simply instilled nasally, only one responded with signs considered as unmistakably due to specific infection. Of the others, one remained free of signs while 4 showed possibly non-specific signs, e.g., rhinitis, conjunctivitis, questionable exanthem.

On the other hand, much better results were obtained in 2 larger groups of children, totalling some 40, who received nasal insufflation of virus finely dispersed with the aid of a special nebulizer. These exhibited signs including rise in temperature, conjunctivitis, Koplik spots and morbilliform exanthem. As in the natural disease, the extent of the signs in individual cases varied somewhat; malaise was very infrequent. In two other groups of children similar findings resulted from intradermal inoculation while the outcome of subcutaneous injections has been less encouraging. It is noteworthy that the characteristic disease picture resembles closely that observed in rhesus monkeys infected with human or egg-passaged virus and is markedly milder than the naturally occurring disease in children, or that produced in the latter by injection of human measles blood.

The influence of edema on the susceptibility of the rabbit to infection with vaccinia. DOUGLAS H. SPRUNT and HAYWOOD TAYLOR. *Depts. of Pathology and Biochemistory of the Duke Univ. School of Medicine, Durham, N. C.* Experiments are described in which it is shown that an increase of the interstitial fluid decreases the susceptibility of the rabbit to infection. This was shown to be true by two types of experiment.

One, the amount of interstitial fluid was increased by multiple intraperitoneal injections of an hypotonic saline solution. The susceptibility of the rabbit to infection with vaccinia was decreased.

Two, administration of the estrogenic hormone to rabbits was found to increase the amount of interstitial fluid. This increase was shown by determination of blood volume and the available fluids of the tissues. The rabbits receiving this hormone were found to be more resistant to infection with vaccinia.

In both of these experiments the spread of particulate matter in the skin was found to be reduced.

It is suggested that the decrease in susceptibility associated with the increased hydration of the

tissues was the result of the virus failing to spread in the tissues. The localization of a virus in the skin exposes fewer susceptible cells and thus decreases the likelihood of viral multiplication. The explanation, however, may not be quite so simple as shifts in the water balance may cause changes in the permeability of the cell and thus result in either an increase or decrease in susceptibility to viral infection.

Reticulum fiber—lymphocyte response in malignant neoplasms. ROBERT J. STEIN. *Pathology Labs., Orleans County Memorial Hospital Newport, Vt.* In malignant neoplasms, whether of slow growth or of the actively progressing variety, when accompanied by stromal lymphoid infiltration exhibit a reticulum fiber hyperplasia. The number of fibers produced is directly proportional to the extent of the lymphoid reaction and independent of the tumor cell activity. Both epithelial and connective tissue tumors from humans and animals exhibit this phenomena. In transplanted mice tumors the sequence of events leading to the first appearance of reticulum fibers was studied. In six to twelve hours after the transplantation an acute inflammatory reaction was evident and characterized by the presence of granulocytes and abundant fibrin formation. After forty-eight to seventy-two hours thin walled capillaries were seen and young fibroblasts with mitotic figures were irregularly dispersed. At about this stage a number of polyblasts were evident. On the third day and thereafter mature appearing lymphocytes were present in the connective tissue stroma and reticulum fibers were able to be demonstrated for the first time. In these series of experiments the presence and quantity of the lymphocytes and the argirophil fibers exhibited no relationship to the rate of growth of the tumor, central necrosis or spontaneous regression of the tumor. Heteroplastic tumor implants on the allantois of chick embryos exhibited a similar lymphocyte-reticulum reaction. Another series of experiments performed to determine whether this phenomena was pathognomonic of tumors or a chronic inflammatory response revealed that such a reaction could be produced by chemical irritants (triglycerides), foreign bodies (sterile sand), and certain bacteria (tubercle bacilli).

The comparative pathology of induced tumors of the salivary glands. PAUL E. STEINER. *Dept. of Pathology, Univ. of Chicago.* To study the causation and histogenesis of "mixed tumors of salivary gland type" seen in man, the salivary glands of animals were exposed to cancerogenic chemicals. Methylcholanthrene was used in mice, rats, guinea pigs, and rabbits, 1:2:5:6-dibenzathracene in mice, and 3:4-benzpyrene in rats. As controls for non-specific irritation cholesterol was used in mice and guinea pigs.

No "mixed tumors" containing epithelium and cartilage were induced, although other types of combined or mixed tumors occurred as follows: adenocarcino-sarcomas, acanthocarcino-sarcomas, and acantho-adenocarcinomas. Such induced tumors do not commonly arise in other tissues. Epithelial tumors encountered were epidermoid cysts, squamous cell carcinomas, and an adenocarcinoma. Epithelial changes which preceded the tumors were squamous metaplasia of acinar and terminal duct cells, and sometimes squamous epithelialization of the pellet space. Connective tissue tumors, spindle and mixed cell sarcomas, showed no differences in morphology and induction time from those induced in other locations. Blood vessel tumors, cavernous hemangiosarcomas, occurred among mice exposed to methylcholanthrene.

Methylcholanthrene in mice induced the widest variety of tumors. Squamous cell carcinomas were easily induced in rats and with more difficulty in guinea pigs. No tumors but early squamous metaplasia (3 days) occurred in rabbits.

The earliest metaplastic cells appeared abnormal, possibly neoplastic. The ease of production of tumors was in marked contrast with the relative absence of spontaneous salivary gland tumors in these animals. The tendency for metaplasia to precede neoplasia and thus determine the type of tumor is again demonstrated; their relationships are important.

Lecithin intravenously: its relationship to the lipoid storage diseases and to the hemolytic anemias. EDNA H. TOMPKINS. *Dept. of Anatomy, School of Medicine, Vanderbilt Univ., Nashville, Tenn.* Injection of lecithin subcutaneously causes local infiltration of macrophages. It was therefore administered intravenously to determine if generalized infiltrations of similar macrophages would ensue comparable to the infiltrations in lipoid storage diseases.

Commercial egg lecithin (Digestive Ferments Company), before and after removal of acetone soluble and ether insoluble impurities and cephalin, was given to rabbits daily for 44 to 74 days. The dosages were increased gradually to maxima daily levels of 0.4 to 1.0 gram. Dosages less than 0.8 grams were well tolerated. The conclusions are based on blood and tissue studies.

The pathologic picture was less that of the lipoid storage diseases, than of the hemolytic anemias, with similarities to the type in sickle cell anemia. Moderate leucocytosis and anemia occurred consistently and monocytosis variably. The spleen and liver were enlarged, the marrow hyperplastic and the adrenals small.

The organs of the reticulo-endothelial system were infiltrated with macrophages which contained whole or fragmented erythrocytes and

stained poorly for unmasked iron, but contains quantities of masked iron. The splenic pulp also contained myelocytes and nucleated red cells, and the nodules were surrounded by homogeneous, acidophilic material.

Detailed blood and urine studies were made in the experiments with purified lecithin. Decreases in erythrocytes, hemoglobin and hematocrit occurred gradually and were checked by sustained reticulocytoses, and accompanied by decrease in volume index and slight increase in icteric index, without change in fragility. The erythrocytes were abnormally malleable. Urine tests were negative for bile pigment and to guiac.

Animal tissue response to metallic magnesium and its alloys. CARL W. WALTER and REUBEN Z. SCHULZ. *Laby. for Surgical Research, Harvard Medical School, Boston, Mass.* Magnesium and its alloys implanted in the subcutaneous tissue, muscle, liver, stomach, mesentery, meninges, and brain of dogs stimulate the formation of a circumscribed envelope of pneuma-granulomatous tissue. The metal becomes jacketed with fibrous tissue surrounded by multilocular and pseudolocular spaces filled with gas under pressure. The lesion expands slowly as gas accumulates, compressing adjacent tissues. An implant near the ureter caused obstruction resulting in aseptic hydronephrosis; in the liver cholangiectasis occurred; in the meninges the convolutions were flattened and there was extensive localized compression and degeneration of the brain. The latter animal had convulsions.

Histologically there is an accumulation of macrophages about scaled fragments of the metal nidus. This mass is surrounded by fibrous tissue containing few or no inflammatory cells. The walls of the locules are thin sheets of fibrous tissue with but few cells and almost no blood vessels. There are usually no inflammatory cells in the spaces. The major inflammatory cell infiltration is seen in the region where the pneuma-granuloma contacts the tissues of the host.

Although the metallic nidus is transparent to x-ray, the pneuma-granuloma can be identified when sufficient gas accumulates.

The implantation of magnesium is a useful experimental method for the production of progressively expansive or constrictive lesions. The pneuma-granuloma is a hazard in industries where accidental implants of magnesium occur. The lesion may be encountered in military surgery as a late complication in those wounded by magnesium containing fragments of bombs or aircraft. Such foreign bodies should be removed immediately to avoid encroachment on vital tissues.

The distribution of radioactive phosphorus administered to leukemic patients. SHIELDS WARREN. *Laby. of Pathology, New England Deaconess Hospital, Boston, Mass.* The distribution of radioactive phosphorus in the organs of eight cases of leukemia has been determined by autopsy. The distribution hinges in part on the degree the leukemia has infiltrated the viscera, the greater the infiltration the heavier the concentration of radioactive phosphorus, but in part depends on selective deposition in the various viscera. Liver, kidneys and bone marrow are among those showing relatively high concentration. A certain amount of radioactive phosphorus is excreted in the bile.

The effect of vitamins, especially pyridoxine and pantothenic acid in preventing sensory neuron degeneration due to nutritional deficiency in pigs. M. M. WINTROBE, M. H. MILLER and R. H. FOLLIS, JR. *Depts. of Medicine and Pathology, Johns Hopkins Univ., Baltimore, Md.* Abnormal gait and sensory neuron degeneration involving peripheral nerves, posterior ganglia, dorsal roots and the posterior columns of the spinal cord, have been produced in young pigs deficient in "B" vitamins other than thiamin. Excellent nutrition and normal development of the nervous system result when pyridoxine, pantothenic acid and choline, as well as riboflavin and nicotinic acid are fed in addition to thiamin. When, of these vitamins, pyridoxine alone is lacking, severe microcytic anemia, epileptiform convulsions, abnormal gait and the changes in the nervous system described above develop. Such changes also occur when only pantothenic acid is lacking and pyridoxine is furnished. In the latter animals alopecia is observed. In both groups poor growth occurs and diarrhea may develop. With the basal diet used, in which casein represented 26 per cent, the omission of choline was associated with the development of changes in the nervous system only in one out of three pigs. These changes were found in the lateral and ventral columns of the spinal cord, thus differing from those observed in other types of deficiency.

This report will summarize observations on 138 pigs raised from the age of three weeks on artificial diets. By the time the report is to be presented further studies concerning the effects of choline deficiency as well as observations dealing with the effects of thiamin, riboflavin, nicotinic acid and A deficiencies on the nervous system will have been completed.

Mammary gland hyperplasia in rats united in parabiosis with gonadectomized partners. ISOLDE T. ZECKWER. *Dept. of Pathology, School of Medicine, Univ. of Pennsylvania, Philadelphia.* Thirty-three female rats were united in parabiosis with ovariectomized partners before 52 days of age. Pituitaries of castrates are known to hypersecrete gonadotropin which passes over to recipient partners and stimulates their ovaries to hypersecrete

estrogen. Progestin is also secreted when the pituitary is present in the recipient. Forty-five biopsies of the mammary glands of the recipients were compared with controls, and 7 pairs were autopsied. Nineteen pairs with controls are alive for further observations. The time intervals studied varied up to 407 days after operation. In 3 weeks marked alveolar hyperplasia occurred; after 7 weeks, lumina of alveoli were greatly distended with secretion, which was grossly visible as milky fluid exuding from cut section; ducts showed no epithelial hyperplasia but were dilated with secretion. Two rats developed circumscribed adenomas. Early the ovaries were enlarged with corpora lutea and ripening follicles, later (when the recipients' pituitaries were enlarged and degranulated) ovaries were composed of granulosa cells with absence of luteinization.

Ten males were united with castrated males. Ten biopsies and 7 autopsies were studied. Androgen secreted in excess by the recipients stimulated the mammary glands to epithelial proliferation with hypersecretion and distention of ducts by secretion, but with less distention of acinar lumina than in females.

The experiments prove that gonads can be stimulated by the pituitary to secrete hormones in sufficient quantities to induce a pathologic hyperplasia of mammary glands. Such studies represent a more natural production of pathologic change than those resulting from intermittently injected exogenous hormones.

THE AMERICAN INSTITUTE OF NUTRITION

NINTH ANNUAL MEETING

Boston, Mass., March 31, April 1, 2, 3, 4, 1942

(For possible corrections in any of the following abstracts see the next issue)

The amino acid composition of animal tissues. ELLIOTT F. BEACH, BERTHA MUNKS and ABNER R. ROBINSON. *Research Laby., Children's Fund of Michigan, Detroit.* A study of the amino acid composition of beef organs and the muscle of various animals has been undertaken to determine (1) their relative dietary value, (2) to what amino acid pattern the growing animal must conform in tissue synthesis, (3) the uniformity or differences in amino acid pattern of muscle protein in all species and classes of animals.

Beef, veal, lamb, pork, chicken, turtle, frog, salmon, cod and shrimp muscle; beef heart, liver, kidney and brain were powdered, after removing lipids and water extractable substances. The preparations contained 16.6 to 14.3 per cent nitrogen.

In the fat-free, water extracted, dry preparations of muscle tissues, total sulfur composed 1.0 to 1.4 per cent; cystine ranged from 1.0 to 1.34 per cent; the methionine contents averaged 3.0 per cent. Tyrosine, tryptophane and phenylalanine were found in relatively constant amounts, ranging from 4.3 to 4.9, 1.2 to 1.4, and 4.0 to 4.7 per cent, respectively. Striking constancy in histidine content was observed (2.2 to 2.4 per cent) while the lysine content varied from 8.2 to 9.7 and arginine from 6.4 to 8.8 per cent.

The tyrosine and histidine contents of beef liver, kidney and brain were approximately the same as those of the muscle tissues, but the liver, kidney and brain preparations contained greater amounts of phenylalanine, tryptophane and cystine (averages were 5.3, 1.7 and 1.8 per cent, respectively) and less arginine and lysine (averages were 6.6 and 6.0 per cent, respectively) than the preparations of muscle tissue.

The prevention by choline, methionine, or casein of dietary cirrhosis of the liver in rats and rabbits. HAROLD BLUMBERG, C. G. MACKENZIE and DAVID SELIGSON. *Dept. of Biochemistry, School of Hygiene and Public Health, Johns Hopkins Univ., Baltimore, Md.* It was previously reported that choline prevented cirrhosis of the liver which was produced in rats fed a diet high in fat and low in protein (casein), with or without *l*-cystine, for 100–150 days. Partial prevention was secured with *d,l*-methionine at a level of 25 mgm. per day, evidently a border-line dosage. In the present investigation the effect of casein level was studied with a choline-deficient diet containing 70 per cent fat. At a 10 per cent casein level all of the rats developed cirrhosis, at 18 per cent most of the rats showed slight cirrhosis, while at 24 per cent cirrhosis was absent. Ten per cent of yeast, in a diet containing 10 per cent of total protein, was also protective. Cirrhosis was produced in young rats on a somewhat similar diet containing 7 per cent casein, 20 per cent fat, and 0.456 per cent *l*-cystine.

Prevention was secured with 0.713 per cent *d,l*-methionine. Methionine was also protective in paired feeding experiments, indicating that difference in food consumption was not an essential factor. Liver lipids were increased in the cirrhotic rats.

Young rabbits developed diffuse nodular cirrhosis of the liver on a choline-deficient diet of the following composition: casein 10, dextrin, 74, lard 10, and salts 6, with supplements of thiamin chloride, riboflavin, pyridoxine, calcium pantothenate, haliver oil, and alpha-tocopherol. Cirrhosis was prevented by a daily supplement of 300 mg. of choline chloride. Liver lipid content of the choline-deficient rabbits was approximately double that of the controls fed choline.

Effect of vitamin A depletion on young adults. SADIE BRENNER and LYDIA J. ROBERTS. *Dept. of Home Economics, Univ. of Chicago, Ill.* A vitamin A deficiency study on six normal adults, four females and two males, was undertaken for the purpose of determining the subclinical signs and their relative order of appearance. The criteria used for judging deficiency were measurements of *1*, dark adaptation on the Biophotometer, Hecht Adaptometer, and Blanchard's Regenometer; *2*, visual fields by perimetry; *3*, changes in the histological structures of the skin as studied in medium punch biopsies of the thigh and by the visibility of blood capillaries; *4*, changes in the conjunctivae studied with a biomicroscope; *5*, changes in the total and differential white blood counts; *6*, vitamin A and carotene content of the blood.

The only observable effect of the deficiency diet even after seven and one-half months for the females and four and one-half months for the males was the decreased blood carotene. The subjects could not be termed depleted by any of the other criteria.

From this investigation it can be concluded that either the subjects did not use their stored vitamin A at a rapid enough rate to cause sufficient depletion or that the criteria used were not subclinical or specific for vitamin A deficiency. The explanation still remains to be found.

The anticataractogenic effect of certain dicarboxylic diamino acids. GLADYS M. COOK and HELEN S. MITCHELL. *Nutrition Laby., Mass. State College, Amherst.* Previous papers from this laboratory have reported the anticataractogenic effect of proteins and of the dicarboxylic-diamino acid fraction of a casein hydrolysate when fed in a cataractogenic ration to young rats. This paper reports the results of testing separately five of the amino acids present in this protective fraction: *aspartic acid, glutamic acid, arginine, lysine* and *histidine.*

Each of these amino acids, respectively, was incorporated into a 25 per cent galactose cataractogenic ration in amounts comparable to that which would be provided by a 15 per cent casein ration. The first three exerted no inhibitory effect when tested in paired feeding experiments in which the control rat received a similar ration containing 5 per cent of casein as the only source of nitrogen. With lysine the average time for cataract development was delayed but the results were irregular and not convincing. With *histidine* the inhibitory action was apparent at the 1 per cent level and more pronounced at the 2 per cent level. Poor food consumption tended to slow down cataractous changes in both rats of a pair. Nevertheless, differences were significant. Attempts to administer histidine by stomach tube or by injection were unsatisfactory.

Since histidine is the only amino acid containing the imidazole nucleus it seemed logical to investigate some other compound containing this radical. Imidazole as such, the only derivative available, proved to be toxic to rats, as previously reported and therefore unsatisfactory for this test. Some less toxic derivative should be investigated.

The production and prevention of hemorrhagic necrosis and cirrhosis of the liver in rats. F. S. DAFT, W. H. SEBRELL and R. D. LILLIE. *Divisions of Chemotherapy and Pathology, National Inst. of Health, Bethesda, Md.* Hemorrhagic necrosis and cirrhosis of the liver in rats on deficient diets have been observed in several laboratories. With a diet containing 4 per cent casein, we have found that suitable variations in the intake of choline and sulfur amino-acids, lead to the production of *a*, a high incidence of hemorrhagic necrosis with no cirrhosis; *b*, a high incidence of cirrhosis with no hemorrhagic necrosis; *c*, a high incidence of both cirrhosis and hemorrhagic necrosis; or *d*, no cirrhosis or hemorrhagic necrosis. Under the conditions of our experiments, choline prevents the cirrhosis, cystine prevents the hemorrhagic necrosis, and methionine prevents both the cirrhosis and the hemorrhagic necrosis. It appears, therefore, that the cirrhosis and the hemorrhagic necrosis are separate and distinct pathological entities.

The effects of zinc deficiency in the mouse. HARRY G. DAY. *Indiana Univ., Bloomington.* Young mice restricted to a diet very deficient in zinc are retarded in growth from the first week. More than 50 per cent die within 3 to 6 weeks. Litter mate controls fed zinc daily (40–60 γ Zn as $ZnSO_4$) thrive about as well as mice on a regular stock diet. The deficient animals suffer loss of hair from the shoulders, back of the neck, and parts of the face. This is not prevented by extra supplements of inositol. The cachectic animals gradually become stuporous one or two days before they die. Food consumption generally is good until two to five days before death.

Catalase activity of liver and kidney is considerably reduced. It is not restored to normal by adding zinc salts to the tissue preparations.

Liver esterase activity is not changed. The content of riboflavin in heart, kidney and liver remains unchanged. Cadmium does not prolong the survival of zinc-deficient mice.

The effect of zinc deficiency on catalase will be discussed.

Biological and chemical studies related to choline deficiency. R. W. ENGEL. *Laby. of Animal Nutrition, Alabama Polytechnic Inst., Auburn.* A choline-deficient diet produced 100 per cent mortality in weanling rats within 10 days. Rats were fed this diet and supplements of beef liver, peanut meal and various strains of yeast, to determine the choline content of these foods. The quantity of supplement necessary to prevent death was used as an endpoint. Five milligrams of pure choline chloride per rat daily was required to protect.

The choline content of the above materials was determined chemically by precipitation as the reineckeate.

Various solvents were tested for their ability to extract choline from yeast. The degree of effectiveness in ascending order was as follows: petroleum ether, ethyl ether, acetone, chloroform, ethanol-ethyl ether (1:1), ethanolskellysolve B (1:1), methanol-chloroform (1:1), ethanol, ethanol-methanol (1:1), and methanol. The chemical analyses, when methanol was used as the solvent, were in good agreement with the results obtained by bioassay.

The choline content of different strains of yeast varies considerably. The choline content of beef liver was approximately twice that of rat liver.

If weanling rats were fed the choline-deficient diet plus choline (20 mgm. per rat daily) for four weeks and the choline was then discontinued, 50 per cent of the animals died within two weeks. The livers of rats which survived for six weeks after the choline was discontinued contained about one third as much choline, as per cent of fresh liver, as did the livers of control rats receiving 20 mgm. of choline per rat daily throughout.

Factors influencing the nutritive value of the tomato. K. C. HAMNER, C. B. LYON, G. H. ELLIS and K. C. BEESON. *U. S. Plant, Soil and Nutrition Laby., Ithaca, N. Y.* More than 2100 tomato plants of an inbred variety have been grown in sand culture with different pure nutrient solutions varying in their content of macro-nutrient elements. Plants were also grown in soil at five locations in the United States and samples of the soils were shipped to Ithaca where plants were grown side by side in each of these soils. The fruits produced have been analyzed for ascorbic acid and are being analyzed for carotene, calcium, phosphorus, and Vitamin B_1.

Variations in the supply of macro-nutrient elements (Ca, K, Mg, N, P and S) resulted in great differences in the growth and fruitfulness of the plants but the ascorbic acid values of fruits varied but little whether the plants produced only one or a great many fruits. In contrast, the location and time of year in which the plants were grown had a marked effect. Fruit produced out-of-doors in the summer had twice the ascorbic acid of comparable fruit from the greenhouse in the winter. This difference was due more to season than to greenhouse conditions as compared to outside conditions. The location where the plants were grown also produced marked effects, some locations producing fruit twice as potent in ascorbic acid as others.

A discussion of other factors affecting ascorbic acid content as well as the effect of various factors on carotene, mineral content and possibly Vitamin B_1 will be given.

Water balance in canine blacktongue. PHILIP HANDLER and W. J. DANN. *Dept. of Physiology and Pharmacology, Duke Univ. School of Medicine, Durham, N. C.* The first symptoms of canine blacktongue are an excess salivation and reddening of the cheeks and gums. These are followed within two weeks by the appearance of ulcerated areas, ropy saliva, complete loss of appetite and the loss of 10–30 per cent of the body weight in three or four days. At this time the animals invariably die.

Since so rapid a loss of weight could only be due to water loss, and also considering the obvious dehydration of these animals, it seemed possible that the immediate cause of death in blacktongue might be improper water balance rather than any impairment of tissue respiratory mechanisms due to cozymase deficiency.

To test this hypothesis physiological saline solution was given subcutaneously to 32 blacktongue dogs just before the appearance of necrotic cheeks. The standard dose was 3 per cent of the body weight daily. This procedure prolonged the lives of 24 dogs, for 25–85 days. In each case there was a considerable initial gain in weight and almost complete restoration of the appetite. In 13 dogs there occurred complete remission of the mouth symptoms for 10–30 days while the remaining eleven showed a partial remission. The animals' weights then gradually declined, their appetites dwindled, the mouth symptoms reappeared and death ensued.

No remedial effect was observed in the other eight dogs.

The results of blood and tissue analyses, performed when death seemed imminent, for the standard constituents and also nicotinic acid, cozymase, and tissue respiration will be presented.

Polyneuropathy in thiamine deficient rats de-

layed by alcohol or whisky. J. V. LOWRY, W. H. SEBRELL, F. S. DAFT and L. L. ASHBURN. *Divisions of Chemotherapy and Pathology, National Inst. of Health, Bethesda, Md.* The effect of the ingestion of alcohol and whisky on the time of onset of polyneuropathy in thiamine deficient rats was investigated. The thiamine was completely withdrawn after a preliminary period of equal and low thiamine intake. The number of days from the withdrawal of the thiamine to the onset of polyneuropathy was determined. Prophylactic and therapeutic experiments were performed to demonstrate the relationship of the thiamine to the polyneuropathy that was used as the criterion of thiamine deficiency. Paired feeding experiments were done to observe: I. The effect of whisky with an isocaloric intake. II. The effect of whisky with an equal intake of the diet. III. The effect of alcohol with an equal intake of the diet. IV. The effect of alcohol and a restricted intake of the diet.

Without exception, the rats on water developed polyneuropathy before the litter mates on alcohol or whisky. In experiment IV polyneuropathy was observed first in the rats on water, next in the rats on alcohol, and last in the rats on alcohol with a restricted intake of the diet. The polyneuropathy observed could be prevented and alleviated by thiamine regardless of whether the rat drank water, alcohol or whisky. In 4 of the rats, that died without having polyneuropathy that was observed, there was an ascites, hydrothorax, and distinctive cardiac pathology.

The development of E deficiency in rabbits and the response to alpha-tocopherol as measured by creatine excretion. C. G. MACKENZIE. *Dept. of Biochemistry, School of Hygiene and Public Health, the Johns Hopkins Univ., Baltimore, Md.* The development of an abnormal creatinuria as the result of avitaminosis E has been studied in 100 young rabbits. The daily creatine excretion increased from the normal level of 10 mgm. or less, to 20 mgm. or more in approximately 3 weeks. The time to abnormal creatinuria was independent of the sex, the gain in weight, and the initial weight over a range of 300 to 800 grams.

The rate and the duration of the response to single oral doses of d,l-α-tocopherol acetate were measured by the temporary fall in urinary creatine. No marked difference was observed in the rate of response following the administration of 2.5 mgm., 5 mgm., or 8 mgm. of the acetate, or 8 mgm. of the acetate plus 5 mgm. of d,l-α-tocopherol. However, the magnitude of the fall in creatine excretion was less at 2.5 mgm. than at the other levels employed.

The average total number of days that the decreased urinary creatine was less than 20 mgm., or the average number of days required for the creatine to once more reach 20 mgm. (counting from the time of treatment) varied with the level of vitamin E fed. In the latter case this ranged from 7.6 days with 2.5 mgm. of vitamin E to 16.4 days with 13 mgm. of the vitamin (8 mgm. of acetate plus 5 mgm. of tocopherol). Considerable variation in the duration of the response was encountered in rabbits receiving the same level of vitamin E.

Vitamin content of rice polishings. WILLIAM M. MCCORD and FRED G. BRAZDA. *Dept. of Biochemistry, The School of Medicine, Louisiana State Univ., New Orleans.* Rice polishings have been analysed by chemical methods in regard to the vitamin content, with most attention being centered on the B group. It has been found that rice polishings are especially high in nicotinic acid and thiamin chloride and that this material is an excellent source for a cheap diet supplement. Certain problems arise, however, in regard to its keeping qualities which must be considered when the material is packed.

The thiamin chloride content is about 25 micrograms per gram, the riboflavin 3.5 micrograms per gram and the nicotinic acid 600 micrograms per gram. Sufficient protein, fat, carbohydrate, iron, calcium and phosphorus are present to make this material an adequate food by itself. The nutritional qualities of the fat and protein in regard to their adequacy are being studied. The content of vitamins E and K is being studied.

Further studies on L. casei ϵ eluate factor in the nutrition of the chick. R. C. MILLS, G. M. BRIGGS, JR., E. B. HART and C. A. ELVEHJEM. *Dept. of Biochemistry, Univ. of Wisconsin, Madison.* Further studies have been made on the rôle of the *L. casei* ϵ factor of Hutchings et al. (B. L. Hutchings, N. Bohonos, D. M. Hegsted, C. A. Elvehjem and W. H. Peterson. J. Biol. Chem. **140:** 681, 1941) in the nutrition of the chick. The basal ration used has the following composition: dextrin 57 grams, casein 18 grams, salt V 6 grams, soybean oil 5 grams, gelatin 10 grams, kidney residue 2 grams, cystine 0.2 gram, inositol 0.1 gram, choline 0.15 gram, thiamine 0.3 mgm., pyridoxine 0.4 mgm., riboflavin 0.4 mgm., Ca pantothenate 1.5 mgm., and nicotinic acid 10 mgm. Each chick received two drops of haliver oil per week. On this ration chicks weigh about 125 grams at four weeks and show a reduced hemoglobin content of the blood and a characteristic shaggy condition of the feathers. Addition of a concentrate of the "eluate factor" increases growth to 230 grams, and produces normal hemoglobin values and feathers. When a biotin concentrate is used in place of kidney residue the addition of 2 per cent solubilized liver extract is necessary to produce maximum growth. A norit eluate from the solubilized liver extract produces normal hemoglobin and

feathers, but only submaximal growth, indicating the presence of at least one other factor in the solubilized liver extract.

The relation between the *L. casei* ϵ factor and factor U, Hogan's anemia factor, and the alcohol precipitate factor of Schumacker and Heuser will be discussed.

Biotin deficiencies in the rat. EDWARD NIELSEN and C. A. ELVEHJEM. *Dept. of Biochemistry, Univ. of Wisconsin, Madison.* Weanling rats placed on a basal ration containing sucrose 73, casein (Labco) 8, commercial egg white 10, salts 4, corn oil 5, choline chloride 0.1 per cent, and receiving the B complex vitamins daily in supplement dishes at the following levels: thiamin hydrochloride 20γ, riboflavin 20γ, pyridoxine 25γ, calcium pantothenate 100γ, and 2 drops of haliver oil per week develop "spectacle eye" at the end of 5 weeks. If the animals are left on this ration for an additional 6 weeks, the following characteristic symptoms develop: eczematous dermatitis, edema of the feet and the mouth, alopecia, and finally spasticity of the hind legs.

Since a similar spasticity has been described in this laboratory resulting from a riboflavin deficiency and aggravated by high fat diets, the relationship of riboflavin and pyridoxine was investigated and found to be without effect, while a high fat ration had some sparing action.

The above described symptoms are prevented or cured with biotin concentrates and biotin. In the curative technic 2–3 weeks are required for the symptoms to completely regress, however, a few cases of severe alopecia required further therapy.

The described synthetic ration allows good growth during the first few weeks, but as the symptoms develop a retardation in growth is noted. When the deficiency is alleviated with biotin supplementation, a growth response was always obtained.

The relation of the symptoms obtained with the egg white ration and the synthesis of biotin in the intestinal tract will be discussed.

The effect of diet on the excretion of pantothenic acid in the urine of equine. P. B. PEARSON and H. SCHMIDT. *Agricultural Expt. Station, Agricultural and Mechanical College of Texas, College Station.* Data have been collected on the daily urinary excretion of pantothenic acid by horses maintained on various dietary regimens. Shetland ponies shortly after weaning at about 8 months of age were fed the experimental diets. The pantothenic acid-deficient diet contained 290 micrograms of pantothenic acid per 100 gm. of feed. The urine was collected quantitatively on 2 consecutive days at monthly intervals from each animal.

Prior to the time the horses were placed on the deficient diet and while they were still on a stock diet consisting of corn, good quality hay and a limited amount of grass the average daily excretion of pantothenic acid was 7.7 mgm. After the animals had been on the deficient diet for approximately 50 days the daily excretion of pantothenic acid had decreased to 0.75 mgm. The average daily excretion of pantothenic acid after 8 to 9 months on the deficient diet was 0.38 mgm. Ponies fed the deficient diet plus approximately 125 grams of brewers' yeast daily were in good condition at the end of 5 months. Their daily urinary excretion of pantothenic acid was 8.1 mgm. There was a definite relationship between the pantothenic acid content of the blood and the amount excreted in the urine. The data indicate that pantothenic acid is a dietary essential for equine.

A study of the ascorbic acid requirements of children of early school age. VIVIAN M. ROBERTS and LYDIA J. ROBERTS. *Dept. of Home Economics, Univ. of Chicago, Ill.* The vitamin C requirements of five children between the ages of 7 and 12 years were studied. To a weighed basal diet providing 15 mgm. of vitamin C, supplements of crystalline ascorbic acid, ranging from 40 to 130 mgm., were added. For each child the amounts of reduced ascorbic acid in the urine and blood plasma were determined on eight levels of intake. Urinary "resting level" and "retentions" and the blood levels were used as criteria for judging the nutrition of each subject in respect to vitamin C.

To further evaluate the state of ascorbic acid saturation of each subject the test dose method was employed. Each 7-day experimental period was terminated by giving 300 mgm. of ascorbic acid and determining the percentage excreted in the urine within 24 hours.

An intake of 65 mgm. for the two youngest children and 75 mgm. for the three oldest was found to be sufficient ascorbic acid to *a*, maintain a blood level above 0.7 mgm. per 100 cc.; *b*, provide for the maintenance of the average "retention" value, and *c*, insure saturation on the basis of a 50 per cent excretion of a 300 mgm. test dose. These levels of intake represent a range of 1.7 to 2.4 mgm. per kgm. of body weight.

Some nutritional requirements of the hamster. JOSEPH I. ROUTH and O. BOYD HOUCHIN. *Biochemical Laby., State Univ. of Iowa, Iowa City.* The hamster is satisfactory for demonstrating various vitamin deficiencies; food consumption is smaller than in the rat, the period of rapid growth is shorter, and the manifestations of deficiency are definite.

Polyneuritis developed within 12 days on a thiamin deficient diet. Oral administration of 3γ daily cured the symptoms within 2 days and produced good growth. Subcutaneous injection was not as effective.

Riboflavin deficiency produced loss of weight and death within 20 days, with variable occurrence of diarrhea and dermatitis around the mouth. With daily oral doses of 4γ the symptoms disappeared and growth was renewed. Injection gave similar results.

On pyridoxin-free diet animals lost weight and died within 24 days. Acrodynia-like dermatitis appeared around the mouth after 12 days. Daily administration of 3γ of pyridoxin cured it in 10 days and produced moderate growth.

When pantothenic acid was lacking, there was loss of weight and a red incrustation around the mouth; death followed within 20 days. Injection of 15γ calcium pantothenate daily produced maintenance; larger doses were necessary for growth. Oral administration was less effective.

Loss of weight and death occurred on a nicotinic acid-free diet. Variable symptoms such as rough stringy fur, denuding, and an abnormal mouth condition were observed. Most of the symptoms disappeared with a daily administration of 100γ.

A synthetic diet containing vitamin-free casein, sucrose, agar-agar, salt mixture, cod liver oil, choline, thiamin, riboflavin, pyridoxin, nicotinic acid, calcium pantothenate, α-tocopherol, inositol, and p-aminobenzoic acid supported moderate growth. Investigation on the fat-soluble vitamins are in progress.

Diet and epithelial hyperplasia in the forestomach of rats and mice. GEORGE R. SHARPLESS. *Dept. of Labs., Henry Ford Hospital, Detroit, Mich.* Hyperplasia of the forestomach epithelium occurs in rats and mice fed diets of either white flour or purified materials low in protein. Four animals fed the latter diet have had a squamous cell carcinoma. Some of the factors necessary to maintain normal epithelium are cystine, nicotinic acid, riboflavine, pyridoxin and choline. The mechanism of lesion formation appears to be irritation of abnormally sensitive epithelium by hair, harsh food particles, pepsin and hydrochloric acid, or bile. Since choline or riboflavine fed alone as supplements will produce more extensive lesions, the action of the protective factors is interdependent. Protection by the supplements is provided by reducing contact between the known irritants and the epithelium and also by increasing resistance of the epithelium to proliferative stimuli.

Metabolism in anorexia nervosa. S. M. SMALL and A. T. MILHORAT. *Depts. of Psychiatry and Medicine, Cornell Univ. Medical College, and the New York Hospital, New York City.* The outstanding somatic change in anorexia nervosa is the extreme weight loss, despite a low basal metabolism. Whether this is due to a metabolic disorder or is the result of inanition has not heretofore been established. Four patients with this condition were maintained on weighed diets for periods as long as five months. The daily excretion of urinary nitrogen, urea, ammonia, preformed creatinine and creatine were determined. During several periods the feces were analyzed for their nitrogen and fat content. In these patients the absorption of protein and fat, the nitrogen balance, and the partition of urinary nitrogenous substances were normal. The amounts of preformed creatinine were proportional to the estimated muscular mass. The absence of creatinuria, and normal retention of ingested creatine were findings similar to those seen in inanition and unlike those noted in weight loss due to hyperthyroidism or in generalized muscular wasting. The caloric intake in all patients was abnormally low. Muscular activity was increased in three patients, and in one patient on whom estimations were made, further increase in activity occurred whenever the caloric intake was augmented. Differences between the caloric intake and total caloric output (basal requirements and estimated activity) could be accounted for by changes in body weight. There was no clinical or chemical evidence of avitaminosis, although the intake of one patient was as low as 600 calories daily. The findings in these patients suggest that the metabolic changes are the result of inanition

Observations on induced riboflavin deficiency in man. R. D. WILLIAMS and H. L. MASON. *Mayo Foundation, Rochester, Minn.* Six women were maintained on a basal diet of foods commonly appearing in American diets. It provided only 0.5 mg. of riboflavin for each 1,000 calories. The diet was supplemented with thiamine hydrochloride, nicotinamide, pyridoxine, calcium pantothenate, choline chloride, inositol, ascorbic acid, vitamins A and D, iron, calcium, phosphorus and iodine. The diet was deficient, so far as it could be judged, only in riboflavin. Two of the subjects received a supplement of riboflavin and, therefore, served in some degree as controls.

The eyes were examined periodically with a slit-lamp microscope and periodic examinations were made of concentrations of protein, calcium, phosphorus, lipids, pyruvate, lactate and glucose in the blood and blood counts. The twenty-four hour excretion of riboflavin was determined once a week and the excretion of riboflavin following a test dose was determined once a month. A fluorometric method was used for determination of riboflavin in food and urine. In the foods, values obtained by the fluorometric method compared favorably with those obtained by a microbiologic assay.

The excretions of riboflavin in twenty-four hours and following the test dose fell progressively. Anemia, impairment of appetite or of gastrointestinal motility, decrease of metabolic rates, abnormalities of carbohydrate metabolism and

psychic disturbances, such as were observed in induced thiamine deficiency, have not been observed in the first six months of this study of riboflavin deficiency. Therefore, many of the signs and symptoms commonly thought to be associated with deficiency of either thiamine, riboflavin or nicotinic acid were not observed in this study of isolated riboflavin deficiency.

Some new dietary essentials required by guinea pigs. D. W. WOOLLEY. *The Rockefeller Inst., New York City.* Guinea pigs failed to grow and soon died when fed a ration of sucrose, casein, corn oil fortified with vitamins, A, D, E and K, thiamin, riboflavin, pyridoxin, nicotinic acid, pantothenic acid, choline, inositol, ascorbic acid, and inorganic salts. No single natural product was found which as ten per cent of the ration converted this mixture to an adequate diet. The following evidence indicated that guinea pigs require at least three dietary essentials besides those now available in pure form. Addition of linseed oil meal produced good growth for about 3 weeks, but following this, failure of growth, loss of weight, and death resulted in the succeeding 2 or 3 weeks. This sequence of events was prevented in most animals by feeding dried grass. Hence dried grass was added to the purified basal ration and the linseed oil meal was fractionated. It was found to contain 2 growth essentials. One was soluble in 50 per cent alcohol and in water but insoluble in pure alcohol or acetone, and removable from solution by fuller's earth. A second growth essential was in the alcohol residue. Addition of either fraction alone to the grass-containing basal ration resulted in no improvement of growth or survival. Addition of both fractions resulted in excellent growth. Neither fraction was replaced by biotin, *p*-amino benzoic acid, gum arabic, or glucurone.

INDEX OF AUTHORS

A

Abbott, L. DeF., Jr. 97
Abramson, D. I. 1
Abreu, B. E. 141, 153
Adams, M. H. 112
Adams, W. L. 141
Ades, H. W. 1
Adler, H. F. 1
Adolph, E. F. 1
Albanese, A. A. 97, 116, 175
Albert, S. 2
Allen, F. M. 17, 173
Allen, J. G. 2, 22, 65
Allen, W. F. 2
Alt, H. L. 20
Altshuler, C. H. 164
Alture-Werber, E. 181
Amberson, W. R. 3, 43
Ambrose, A. M. 141, 142
Anchel, M. 140
Anderson, C. E. 97
Anderson, H. H. 142, 146, 160
Anderson, R. 22
Anderson, R. C. 37
Andrews, H. L. 142
Andrews, J. C. 97, 98
Angerer, C. A. 3
Angerer, H. 3
Ansbacher, S. 98
Appel, M. 183
Archibald, R. M. 67, 139
Armstrong, W. D. 98
Arnett, V. 3
Artom, C. 99
Ashburn, L. L. 190
Asher, R. 44
Astwood, E. B. 4, 86
Atcheson, G. H. 4
Atkinson, A. J. 1
Auerbach, O. 170
Aumann, K. W. 96
Axelrod, A. E. 108
Axelrod, B. 99

B

Bachmann, G. 4
Baer, A. 81
Baetjer, A. M. 5
Bailey, J. H. 114
Bair, H. L. 79
Baker, R. D. 173
Baldes, E. J. 23
Balfour, W. M. 174
Balls, A. K. 99
Barborka, C. J. 25
Barbour, J. H. 143
Bard, P. 11, 55
Barkan, G. 143
Barker, S. B. 5
Barnes, B. 5
Barnes, F. W. 99
Barnes, R. H. 137
Barnes, T. C. 6
Barnum, C. P. 98
Barrera, S. E. 65
Barrett, C. D., Jr. 174
Barstow, R. 40
Barton, C. F. 11, 46
Batterman, R. C. 143
Baudisch, O. 99
Bauer, C. D. 100
Bauman, K. L. 132
Baumann, C. A. 100
Baxt, J. O. 23
Beach, E. F. 187
Bean, J. W. 6, 8, 92
Becker, R. F. 7, 93
Becker, T. J. 143, 165
Beckman, H. 144
Beeson, K. C. 189
Bennett, H. S. 144
Bennett, M. A. 100
Berg, C. P. 100, 102
Berg, R. 140
Bergmann, M. 112
Berkman, S. 108
Bernhart, F. W. 101
Bernheim, F. 101
Berry, G. P. 183
Beuding, E. 30
Beutner, R. 144, 145
Beyer, K. H. 7
Bier, O. G. 178
Bischoff, F. 101
Blair, H. A. 7
Blair, J. R. 8
Blankenhorn, J. 174
Blankenhorn, M. A. 174
Bloch, H. S. 125
Bloor, W. R. 123
Blum, H. F. 8
Blumberg, H. 187
Boals, R. T. 36
Bodansky, O. 101, 121
Bohr, D. F. 8
Bollman, J. L. 109, 174
Borchers, R. 102
Borduas, A. 8
Bourque, J. E. 9
Bowman, D. E. 102
Boyd, E. M. 145
Boyd, M. J. 102
Boyd, T. E. 9
Bozler, E. 9
Brady, J. M. 44
Branch, C. F. 175
Brand, E. 103
Brassfield, C. R. 10, 29
Braun-Menendez, R. 10
Brazda, F. G. 103, 190
Brenner, S. 188
Brewer, G. 27
Briggs, G. M., Jr. 190
Brink, F., Jr. 19
Britton, S. W. 10, 41
Brobeck, J. R. 10, 76, 84
Bronfenbrenner, J. 179
Brooks, C. 145
Brooks, C. McC. 11, 50
Browne, J. S. L. 41, 139
Bruce, F. 172
Bruger, M. 109
Bruhn, J. M. 11, 46
Brush, B. 36
Bucher, G. R. 11
Buchter, J. A. 162
Bueding, E. 12
Burns, E. L. 175
Burrill, M. W. 12, 33
Buschke, W. 175
Butts, J. S. 103

C

Cahill, W. M. 104
Cain, C. K. 119
Calesnick, B. 144, 145
Calkins, V. P. 104
Campbell, B. 35
Campbell, J. 146
Campbell, W. W. 104
Canavarro, K. de S. 92
Cantarow, A. 12, 69
Canzanelli, A. 13
Carlson, G. R. 31
Carmichael, E. B. 13, 14, 46, 67
Carr, C. J. 147
Cassels, D. E. 126
Cattell, McK. 146
Chaffee, E. 125
Chambers, R. 14
Chambers, W. H. 5
Chapin, M. A. 175, 183
Charipper, H. A. 18, 30
Chase, H. F. 157, 172
Chen, K. K. 14, 37
Childs, A. 105
Chin, Y. C. 146
Chow, B. F. 33
Ch'uan, T. K. 146
Chunn, C. F. 36
Ciereszko, L. S. 105
Climenko, D. R. 15
Clark, B. B. 141, 147
Clark, D. B. 85
Clarke, E. 14
Clarke, G. J. 101
Clausen, S. W. 105
Code, C. F. 15, 39
Cohen, A. 145
Cohen, S. S. 105
Cohn, E. T. 106
Cole, K. S. 15, 47
Cole, V. V. 147
Collard, J. 129
Collins, D. A. 16, 35
Coman, D. R. 176
Comroe, J. H., Jr. 16, 90
Cone, N. M. 147
Conklin, R. E. 16, 24, 30
Conn, E. S. 106
Conn, J. W. 106
Cook, G. M. 188
Coolidge, T. B. 106
Coombs, H. C. 17, 147
Cooper, G. R. 127
Cope, O. M. 17
Corbin, K. B. 93
Corcoran, A. C. 17
Cornatzer, W. E. 98
Cortell, R. 148
Coryell, C. D. 111
Cosby, R. S. 32, 91
Cowgill, G. R. 132
Cox, W. M., Jr. 107
Crandall, L. A., Jr. 18
Csonka, F. A. 107
Cullen, M. L. 78
Culler, E. A. 18
Cunningham, R. W. 148, 151

D

Daft, F. S. 188, 190
Dahlstet, J. 154
D'Angelo, S. A. 18
Dann, F. P. 107
Dann, W. J. 189
Darrow, C. W. 19
David, N. A. 148
Davies, D. F. 110
Davies, P. W. 19

Davis, H. 27
Davis, J. E. 19
Davson, H. 19
Dawson, P. M. 39
Day, H. G. 188
Deeley, S. E. 96
de Espanes, E. M. 148
Deichmann, W. 148
Demarsh, Q. B. 20
Dille, J. M. 149
Dillon, J. B. 20
DiPalma, J. R. 20
Dische, Z. 107
Doisy, E. A. 119
Donahue, D. D. 149, 170
Dorfman, A. 108
Dosne, C. 8, 21
Dotti, L. B. 21
Dow, P. 21, 36
Dow, R. S. 22
Dragstedt, C. A. 149
Dragstedt, L. R. 2, 22, 65
Draize, J. H. 149
Dresbach, M. 22
Dripps, R. 150
Drips, D. G. 128
Driver, R. L. 22
Drury, D. R. 23
Dumke, P. 150
Dumke, P. R. 90
Dunn, M. S. 111
Dworkin, S. 23
Dye, J. A. 5
Dyniewicz, H. 168

E

Eagle, H. 154, 176
Eaton, M. D. 176
Eckstein, R. W. 34
Edwards, J. E. 140
Eichelberger, L. 105
Eichna, L. W. 150
Elderfield, R. C. 14
Elgart, S. 23, 31, 61
Elliott, K. A. C. 108, 122
Ellis, G. H. 108, 189
Ellis, S. 150
Elvehjem, C. A. 108, 133, 190, 191
Engel, R. W. 189
Ensor, C. 4, 80
Enzer, N. 81
Erickson, C. C. 176
Erickson, J. O. 127
Escobar, R. A. 151
Essex, H. E. 23
Ets, H. N. 24
Ettinger, G. H. 77
Evans, E. A., Jr. 109
Evans, W. E., Jr. 151
Eversole, W. J. 28
Eyster, J. A. E. 24, 30

F

Falk, K. G. 121
Fassett, D. W. 155
Fazekas, J. F. 12, 40
Feindel, W. H. 90
Feinstein, R. N. 50
Felder, R. E. 1
Fellows, E. J. 148, 151
Fenn, W. O. 24
Field, J., 2nd 27, 58
Field, J. B. 100
Fierst, S. M. 1
Finnegan, J. K. 151
Fisher, A. M. 133
Fisher, K. C. 24
Flacks, K. 1
Fleischmann, W. 24
Flexner, J. 109
Flexner, L. B. 28
Flock, E. V. 109
Floyd, N. F. 125
Fogg, L. C. 177
Folch, J. 110
Follis, R. H., Jr. 175, 186
Foltz, E. 25
Ford, Z., Jr. 137
Forman, S. E. 156
Fournier, G. 25
Fraser, T. A. 60
Free, A. H. 110
Freeman, L. W. 25, 51
Freeman, N. E. 78
Freeman S. 26, 42, 47
French, C. R. 10
Freund, J. 178
Friedberg, L. 24, 26
Friedemann, T. E. 110, 111
Frieden, E. H. 111
Friedgood, H. B. 26
Friedman, M. M. 111
Fries, E. C. 39
Frost, D. V. 107
Fruton, J. S. 112
Fu, S. C. 167
Fuerstner, P. G. 151
Fuhrman, F. A. 27, 58
Fulton, G. P. 52
Furchgott, R. F. 27
Futcher, P. H. 67

G

Gaffron, H. G. 112
Galambos, R. 27
Gales, J. W. 167
Gantt, W. H. 28, 74
Gardner, J. 129
Gaunt, R. 28
Gavin, G. 124
Gellhorn, A. 28
Gellhorn, E. 3, 28, 46
Gemmill, C. L. 29
Gerard, R. W. 29, 31, 85, 134
Gesell, R. 10, 29, 36, 58
Gibbs, E. L. 29
Gibbs, F. A. 29
Gibson, R. B. 131
Gildea, E. F. 123
Gilson, L. E. 112
Gilson, W. E. 24, 30
Girden, E. 30
Glaser, G. H. 92
Godfrid, M. 119
Goebel, W. F. 112
Gold, H. 151, 170
Goldfarb, W. 30
Goodman, H. C. 88
Gordon, A. S. 18, 30
Goudsmit, A. 31
Grady, H. G. 8
Graef, I. 69
Graham, J. 29, 31
Graubard, M. 31
Gray, J. S. 37, 78
Grayman, I. 31
Green, H. D. 32, 33
Green, M. W. 152, 158
Greenberg, D. M. 106
Greene, R. R. 12, 33
Greengard, H. 12, 59, 72
Greenstein, J. P. 113
Greenwald, I. 113
Greep, R. O. 33
Gregg, D. E. 34, 68, 80
Gregory, R. A. 15
Greiber, M. F. 156
Greisheimer, E. M. 34
Griswold, R. A. 142
Grollman, A. 34
Gross, J. 23
Grundfest, H. 35
Gunning, R. E. 80
Gruzhit, O. M. 177
Gurchot, C. 152
Gurin, S. 114
Gustafson, G. E. 110

H

Haag, H. B. 49, 152
Hadidian, Z. 158
Haege, L. F. 24
Hafkesbring, R. 34
Hahn, P. F. 177
Hailman, H. 46
Haldi, J. 4
Hall, F. G. 35
Hallenbeck, G. A. 15
Halliday, D. 134
Hamilton, A. S. 16, 35
Hamilton, J. G. 163
Hamilton, P. 139
Hamilton, P. B. 67
Hamilton, P. G. 38
Hamilton, W. F. 21, 36, 172
Hamner, K. C. 189
Handler, P. 189
Handley, C. A. 153
Hanke, M. E. 114
Hansen, E. T. 29, 36
Hardwicke, H. M. 7
Harkins, H. N. 36
Harkness, D. M. 114
Harned, B. K. 147
Harris, A. S. 35, 37
Harris, J. S. 47
Harris, P. N. 37
Harris, S. C. 37
Harrison, T. R. 34
Hart, E. B. 190
Hart, W. M. 38
Hartley, G., Jr. 177
Harvey, E. N. 38
Hass, G. M. 178
Haugen, G. E. 111
Hayes, G. J. 38, 96
Heggen, G. 99
Heidelberger, M. 178
Heilbrunn, L. V. 38
Heinen, J. H. 79
Hellebrandt, F. A. 39
Helmer, O. M. 17, 114
Helmholz, H. F., Jr. 39
Hemingway, A. 115, 137
Henny, G. 83
Henny, G. C. 135
Herrick, J. F. 23, 39
Herrin, R. C. 40
Herrlich, H. 40
Herrmann, J. B. 153
Hertig, A. T. 178
Hertzman, A. B. 20, 75
Hess, W. C. 115, 137
Hildebrand, G. J. 63
Hill, H. C., Jr. 73
Hiller, A. E. 67
Himmelsbach, C. K. 153
Himwich, H. E. 12, 40
Hine, C. H. 153, 156
Hines, H. M. 49, 85
Hirschmann, H. 115
Hisey, A. 3, 43
Hitchcock, D. I. 115
Hitchings, G. H. 116
Hodes, R. 40
Hodge, H. C. 123
Hoefer, P. F. A. 41
Hoehn, W. M. 133
Hoff, H. E. 45, 62, 76, 94
Hoffman, M. M. 41, 139
Hoffman, W. S. 116
Hogan, R. B. 154
Holck, H. G. O. 154
Holland, C. G. 41
Hollander, F. 116, 118
Holman, J. H. 153
Holman, R. L. 179
Holt, J. P. 42
Holt, L. E., Jr. 116, 117
Hong, J. 42, 63
Horwitt, M. K. 117, 134
Houchin, O. B. 117, 191
Hough, V. H. 42
Howard, H. W. 137
Huggins, C. 79
Hughes, H. B. 133
Hurwitz, M. 26

I

Ice, G. 172
Ingelfinger, F. J. 43
Irvin, J. L. 118
Ivy, A. C. 25, 59, 72

J

Jablons, B. 179
Jacobs, J. 172
Jacobs, J. E. 3, 43
Jacobson, E. 43
Jahn, T. L. 6
Jailer, J. W. 135
Janota, M. 183
Jemerin, E. E. 116, 118
Joachim, K. 43
Johnson, C. A. 88
Johnson, J. F. 44
Johnson, V. 25, 51, 80
Johnston, M. W. 106
Jones, H. 182
Joseph, M. 152
Jung, F. T. 44

K

Kabat, E. A. 118
Kajdi, C. 97, 116
Kalckar, H. M. 119
Kalmanson, G. M. 179
Kamin, H. 120
Karpovich, P. V. 44
Karr, N. W. 154
Karstens, A. I. 96
Kassell, B. 103
Katz, G. 154
Katz, L. N. 44
Katz, Y. J. 114
Katzman, P. A. 119
Kaufman, W. 45, 62
Kaulbersz, J. 45
Kaulbersz, J. W. 47
Kay, F. A. 14, 46, 67
Keith, N. M. 180
Keller, A. D. 8, 11, 46
Kemmerer, K. S. 107
Kempf, G. F. 114
Kempner, W. 46, 78
Kendall, E. C. 28, 119
Kent, J. D. 34
Kenyon, A. T. 155
Kepler, E. J. 128, 130
Kessler, M. 28, 46
Kies, M. W. 99
Killian, S. 44
King, B. G. 47
King, H. E. 180
King, R. E. 160
Kirby-Smith, J. S. 8
Kitzmiller, K. V. 148
Klaus, R. K. 81
Klein, J. R. 120, 132
Kleinberg, W. 30
Kleiner, I. S. 21
Kline, R. F. 10, 41
Kmieciak, T. C. 110, 111
Knowlton, K. 155
Koehler, A. E. 120
Kohlstaedt, K. G. 114
Kohn, H. I. 47
Kolin, A. 155
Kopeloff, L. M. 65
Koppanyi, T. 152, 155, 158
Koser, S. A. 108
Kosman, A. J. 47
Kotalik, G. 56
Kozelka, F. L. 153, 156
Krantz, J. C., Jr. 156
Krayer, O. 156
Krieger, H. P. 88
Krop, S. 156
Kryter, K. D. 51
Kubin, R. 148
Kwit, N. T. 152
Kyker, G. C. 120

L

Lambert, E. F. 11
Lambert, E. H. 47
Lamport, H. 48
Landau, R. L. 156
Landis, C. 48
Landow, H. 118
Landy, M. 174
Langley, L. L. 48
Larrabee, M. G. 40
Larsen, E. M. 39
Larson, P. S. 49, 152
Laug, E. P. 149
Lavine, T. F. 120
Lawrence, J. H. 163
Lawrence, W. E. 11, 46
Lawson, H. 49
Lazere, B. 49, 85
Leake, C. D. 157, 160
Le Blond, C. P. 49
Lebond, C. 123
Lee, W. V. 64
Lehman, A. J. 157, 172
Lehman, R. A. 157
Lein, A. 50
Lemon, H. M. 126
Lenox, W. G. 29
Leonard, W. E. 50
Leonards, J. R. 110
Levine, P. 180
Levine, R. 50
Levine, V. E. 121
Lewis, J. M. 121
Lewis, L. A. 121
Lewis, R. N. 32, 33
Li, R. C. 158
Libet, B. 108, 122
Licklider, J. C. R. 51
Lillie, R. D. 188
Linegar, C. R. 155, 158
Link, K. P. 100
Lipmann, F. 122
Lipschitz, W. L. 158
Lipscomb, A. 18
Lisco, H. 180
Liston, L. 54, 55
Lloyd, D. P. C. 51
Loew, E. R. 9

Loewy, A. 25
Logan, M. A. 102, 112
Long, C. N. H. 10, 84
Longenecker, H. E. 122
Longini, J. 51
Louis, L. 31
Lowell, F. C. 180
Lowry, J. V. 190
Lowy, K. 51
Lu, C.-s. 162
Lucké, B. 181, 183
Luduena, F. P. 160
Ludwick, T. M. 66
Lutz, B. R. 52
Lyon, C. B. 189

M

MacFadyen, D. A. 139
Macht, D. I. 53, 54, 55
Macht, M. B. 55, 56
Mackenzie, C. G. 122, 187, 190
Mackenzie, J. B. 122
MacLachlan, P. L. 123
Maclean, A. R. 39
Maddock, S. 181
Maes, J. P. 56, 65
Maison, G. 56
Maison, G. L. 163
Majovski, G. J. 169
Man, E. B. 72, 123
Manery, J. F. 57
Mann, F. C. 23, 39
Mann, W. 123
March, D. G. 143
Marchello, A. 25
Maris, E. P. 184
Marmont, G. 15
Marrazzi, A. S. 57, 159
Marsh, D. F. 159
Marshak, A. 57
Marshall, W. H. 57
Martin, A. W. 58
Martin, G. J. 58, 98
Martin, S. J. 159
Martin, W. P. 176
Mason, A. 29, 58
Mason, H. L. 123, 192
Mason, M. F. 124
Masor, N. A. 59
Masson, G. 59
Mast, S. O. 59
Mathieson, D. R. 154
Mattil, K. F. 122
Mattill, H. A. 104
Mattis, P. A. 159
Maurer, J. 136
Mautz, F. R. 114
Mayer, M. 178
Mayerson, H. S. 59
Maynard, L. A. 108
McCawley, E. L. 151, 160, 168
McChesney, E. W. 52
McCoord, A. B. 105
McCord, W. M. 190
McCoy, R. H. 124
McCullagh, D. R. 101
McCullagh, E. P. 121
McCuire, G. 121
McHenry, E. W. 124
McIntire, F. C. 129
McIntyre, A. R. 160
McIvor, B. 160
McKee, C. M. 181
McKibbin, J. M. 133
McLain, P. L. 52, 162
McMeekin, T. L. 125
McPhail, M. K. 160
Medes, G. 125
Meek, W. J. 24, 64
Mehl, J. W. 125
Mellish, C. H. 162
Mellors, R. C. 114
Member, S. 109
Mendenhall, W. L. 161
Mendez, R. 161
Mercer, T. 171
Merlis, J. K. 60
Mertz, E. T. 125
Meschan, I. 68
Mettler, F. A. 61
Meyer, K. 125
Miley, G. 61
Milhorat, A. T. 192
Miller, B. F. 126
Miller, J. W. 170
Miller, M. H. 186
Miller, Z. B. 126
Millman, N. 44
Mills, R. C. 190
Mirsky, I. A. 23, 31, 61
Mitchell, H. S. 188
Modell, W. 101, 170
Moe, G. K. 95, 161
Moetsch, J. C. 131
Moore, D. H. 118
Moore, R. C. 107
Moreland, F. B. 126, 127
Morgareidge, K. 127
Morrison, J. L. 161
Morse, M. 126
Moss, R. E. 43
Moss, W. G. 80
Mrazek, R. 61
Mueller, A. J. 107
Mullins, L. J. 61
Munks, B. 187
Muntwyler, E. 114
Myers, V. C. 110

N

Nachmansohn, D. 62
Nahum, L. H. 45, 62
Najjar, V. A. 117
Necheles, H. 62, 64
Nelson, D. 62
Nelson, N. 23, 31, 61
Nelson, W. O. 63, 79
Neurath, H. 127
Nicholson, H. C. 42, 63
Nickerson, N. 90
Nielsen, E. 134, 191
Nier, A. O. 115, 137
Nims, L. F. 29
Nixon, E. N. 74
Novak, C. G. 61
Nuhfer, P. A. 162

O

Oberst, F. W. 127
Ogden, E. 63
Olander, G. A. 88
Olcott, H. S. 128
Olson, W. H. 62, 64
Opdyke, D. F. 72
Oppenheimer, E. T. 47
Oppenheimer, M. J. 64
Orten, A. U. 128
Orten, J. M. 128
Orth, O. S. 64
Oster, R. H. 86
Osterberg, A. E. 128, 180
Otto, H. 151
Overman, R. S. 100
Owens, F. M., Jr. 2, 22, 65

P

Pace, D. M. 59
Pacella, B. L. 65
Paff, G. H. 157
Page, E. W. 63
Page, I. H. 17, 114
Pallares, D. S. 35, 37
P'an, S-y. 162
Pappenheimer, J. R. 56, 65
Parsons, H. T. 129
Paschkis, K. 69
Paschkis, K. E. 65
Pastorius, G. J. 52, 162
Patras, M. C. 9
Patterson, T. L. 66
Patton, H. D. 76
Paul, W. D. 132
Pearlman, W. H. 66
Pearson, P. B. 191
Pecher, C. 163
Peoples, S. A. 163
Perkins, P. 112
Perlow, S. 44
Perlzweig, W. S. 133
Peters, R. 115
Petersen, W. E. 66
Peterson, W. H. 129
Pfeiffer, C. 163
Phatak, N. 148
Philips, F. S. 129
Phillips, G. W. 14, 46, 67
Phillips, R. A. 67
Pike, F. H. 148
Pincus, G. 31, 66, 67
Pitts, R. F. 67
Plummer, A. J. 164
Polskin, L. J. 129
Pommerenke, W. T. 182
Ponder, E. 68
Pool, J. L. 41
Popper, H. 168
Power, M. H. 130
Pritchard, W. H. 34, 68
Purdy, M. 172

Q

Quick, A. J. 130
Quigley, J. P. 68

R

Racker, E. 69
Radzow, K. H. 32, 33, 68
Raiziss, G. W. 131
Rake, G. 182, 184
Rakoff, A. E. 69
Ralli, E. P. 69, 76
Ramsey, R. W. 70
Randell, E. 57
Rankin, A. D. 70
Rapport, D. 13
Ratnoff, O. D. 4
Read, H. C. 160
Read, M. R. 68
Reed, B. P. 70
Reed, C. I. 61, 70
Rehm, W. S. 70
Reinecke, R. M. 131, 132
Remmert, LeM. F. 103
Renshaw, B. 71
Rice, J. C. 103
Rich, E. 40
Richards, R. K. 71, 148
Richardson, A. P. 164
Riddle, O. 72
Riggs, D. S. 72
Riker, A. J. 129
Ring, G. C. 72
Rivera, G. 164
Roback, R. A. 72
Robbie, W. A. 131
Roberts, L. J. 188, 191
Roberts, V. M. 191
Robinson, A. R. 187
Robinson, F. J. 128, 130
Robinson, S. 73
Robinson, T. W. 73
Rock, J. 178
Rocha e Silva, M. 149
Rodbard, S. 73
Rogoff, J. M. 74
Rojas, A. G. 35, 37
Roos, A. 74
Rosen, V. H. 74
Rosenberg, S. 164
Rosenfeld, M. 176
Rosenow, E. C. 182
Rosenthal, S. R. 182
Ross, J. F. 175, 183
Ross, W. F. 131
Roth, G. M. 75
Roth, L. W. 75
Routh, J. I. 191
Rubenstein, B. B. 75
Rubin, J. 112
Rubin, M. A. 76
Rubin, S. H. 76
Ruch, T. C. 76
Rudolph, G. G. 104
Ruhe, C. H. W. 52
Russell, J. A. 77
Ryer, R. 77

S

Sacks, J. 164
Sah, P. P. T. 142
Salit, P. W. 132
Salomon, K. 132
Sample, A. B. 98
Samuels, L. T. 132, 164, 169
Sandblom, J. P. 59
Sandiford, I. 155
Sandow, A. 77
Sandweiss, D. J. 66
Saphir, O. 183
Sapirstein, L. A. 128
Sarett, H. P. 132
Sawyer, M. E. M. 77
Sayers, G. 128
Sayers, M. 128
Schaefer, A. E. 133
Schecter, A. E. 78
Schein, A. H. 131
Schenken, J. R. 175
Scherp, H. W. 183
Schiffrin, M. J. 78
Schlayer, C. 78
Schlumberger, H. 181, 183
Schlutz, F. W. 126
Schmidt, C. F. 16
Schmidt, C. L. A. 138
Schmidt, G. 181
Schmidt, H. 191
Schmidt, L. H. 133
Schoenheimer, R. 99
Schroeder, E. F. 68
Schubert, C. 169
Schultze, M. O. 124
Schulz, R. Z. 186
Scott, D. A. 133
Scott, D. B. McN. 108
Scott, W. W. 79
Scruggs, W. 62
Seager, L. D. 164
Searcy, H. B. 145
Sebrell, W. H. 188, 190
Seegers, W. H. 184
Seevers, M. H. 143, 166
Segaloff, A. 79
Selle, R. M. 165
Seltzer, J. I. 52
Selye, H.
2, 8, 14, 21, 25, 59, 79, 94
Severac, M. 131
Sexton, E. L. 125
Shaffer, J. M. 165
Shaffer, M. F. 184
Sharp, D. G. 127
Sharpless, G. R. 192
Shaw, D. R. 183
Shea, P. C. 92
Sheard, C. 75, 79
Shedlovsky, T. 112
Shelton, R. S. 143, 165
Sherman, A. W. C. 134
Shettles, L. B. 116
Shideman, F. E. 166, 169
Shipley, R. E. 34, 68, 80
Shock, N. W. 80, 167
Shorr, E. 27
Shuler, R. H. 80, 154
Shull, G. M. 134
Shumacker, H. B., Jr. 24
Sichel, F. J. M. 38
Silvette, H. 80, 166
Simister, T. H. 16
Simon, B. E. 129
Simon, F. P. 134
Simonson, E. 81
Sinclair, R. G. 137
Sklersky, S. 129
Slaughter, D. 167
Sloan, H. 81
Slotin, L. 109
Small, S. M. 192
Smirnow, A. E. 123
Smith, E. L. 88, 154
Smith, K. U. 81
Smith, P. K. 76, 94
Smith, R. G. 167
Smythe, C. V. 134
Snider, R. S. 82, 84
Snodgrass, J. M. 82
Snyder, F. F. 82
Sobel, A. E. 129
Solandt, D. Y. 57
Soley, M. H. 167
Somogyi, M. 135
Soong, H. Y. 167
Soskin, S. 50
Spencer, E. C. 54, 55
Sperry, W. M. 135
Spiegel, E. 83
Spiegel, E. A. 64
Spiegel-Adolf, M. 83, 135
Spiridonoff, S. V. 168
Sprunt, D. H. 184
Stanley, W. M. 105
Stannard, J. N. 83
Steggerda, F. R. 83
Steigmann, F. 168
Stein, R. J. 185
Steiner, P. E. 185
Steinhardt, J. 136
Stekol, J. A. 136
Stern, K. G. 136
Stewart, W. C. 137
Stickney, J. C. 83, 87
Stinger, D. 2, 65
Stokes, J., Jr. 184
Stotz, E. 140
Stowell, A. 82, 84
Stoyanoff, V. A. 135
Strait, L. A. 151, 168
Straus, W. L., Jr. 24
Street, S. F. 70
Strong, F. M. 129

Struck, H. C. 70
Sullivan, M. X. 115, 137
Summers, P. 78
Summerson, W. H. 137
Sure, B. 137
Svirbely, J. L. 149
Swan, K. C. 132
Swanson, M. 99
Sweet, W. H. 144
Swendseid, M. 115
Swendseid, M. E. 137
Swim, J. B. 168
Szego, C. M. 169

T

Talbot, S. A. 84
Tarver, H. 138
Tashiro, S. 138
Taube, H. 150
Taylor, H. 184
Taylor, J. D. 123
Templeton, R. D 24
Teply, L. J. 108
Tepperman, J. 10, 77, 84
Thannhauser, S. J. 181
Theisen, J. K. 166, 169
Thienes, C. H. 151, 169
Thompson, C. R. 143
Thomson, J. D. 49, 85
Titus, H. W. 107
Tobias, J. M. 85
Tocantins, L. M. 85
Toennies, G. 100, 138
Toman, J. E. P. 86
Tompkins, E. H. 185
Torda, C. 169
Tracy, A. H. 131
Travell, J. 170
Treat, A. E. 92
Treffers, H. P. 178
Tripp, J. T. 174
Truax, F. L. 123
Tum-Suden, C. 86
Turrell, E. S. 86
Tweedy, W. R. 104
Tyler, D. B. 87
Tyslowitz, R. 4, 86
Tytell, A. A. 112

U

Upholt, H. V., Jr. 44

V

Vaichulis, J. A. 170
Valaer, P. J. 149, 170
Van Campen, M. G. 165
Van Dyke, H. B. 33
Van Harreveld, A. 87
Van Liere, E. J. 83, 87
Van Loon, E. J. 147
Van Norman, R. 154
Van Slyke, D. D. 67, 139
Vennesland, B. 109
Venning, E. H. 139
Vermeulen, C. 22
Vinacke, W. E. 48
Vintinner, F. J. 5
Virtue, R. W. 139
Voegtlin, W. L. 59
von Oettingen, W. F. 149, 170

W

Waelsch, H. 139
Wainio, W. W. 87
Wakerlin, G. E. 47, 88
Wald, G. 88
Walker, B. S. 140
Walter, C. W. 186
Walton, C. 173
Walzl, E. M. 88
Wang, S. C. 88
Wangerin, D. M. 97, 116
Warkentin, J. 89
Warren, C. O. 89
Warren, M. R. 143, 165
Warren, S. 123, 177, 180, 186
Wastl, H. 89
Watt, J. G. 90
Wearn, J. T. 34, 80, 116
Weatherby, J. H. 171
Wedd, A. M. 7
Wégria, R. 90
Weichselbaum, T. E. 135
Weinerman, A. 129
Weinstein, V. A. 116, 118
Weir, E. G. 90
Weir, J. R. 88
Weisberger, A. S. 68
Welch, A. D. 156, 159, 171
Welch, C. S. 141
Welch, E. A. 123
Weld, C. B. 90
Wells, H. S. 91
Wells, J. A. 149, 171
Werch, S. C. 111
Werle, J. M. 68, 91, 92
Wertenberger, G. E. 34
Werthessen, N. T. 91
Westerfeld, W. W. 140
Weston, K. 64
Whatmore, G. B. 149
Wheeler, R. E. 124
White, A. 105
White, J. 140
Whitehorn, W. V. 92
Wiggers, C. J. 91, 92
Wiggers, H. C. 92
Wigginton, C. 93
Wilde, W. S. 93
Wilkins, H. F. 151
Williams, E. G. 171
Williams, J. R., Jr. 34
Williams, R. D. 123, 192
Wilson, D. W. 114
Winder, C. V. 44, 93
Windle, W. F. 7, 20, 93
Windsor, E. 120
Winfield, J. M. 45
Winkler, A. W. 76, 94
Winter, H. 94
Wintrobe, M. M. 186
Wisansky, W. A. 98
Wolf, A. V. 94
Wolf, S. 95
Wolff, H. G. 95
Wood, E. H. 95
Woodbury, R. A. 141, 172
Woolley, D. W. 193
Woolsey, C. N. 38, 88, 96
Wortis, J. 96
Worzniak, J. J. 36
Wyman, L. C. 86
Wynn, W. 4

Y

Yonkman, F. F. 172, 173
Youland, W. E. 173
Youmans, W. B. 96

Z

Zeckwer, I. T. 186
Zweifach, B. W. 14

INDEX OF SUBJECTS

A

Absorption from the rumen 70
Absorption, percutaneous, of sulfides 149
Acetins, tolerance to 158
Acetylcholine and eserine, action of 36
Acetylcholine, cH and stimulating action of 58
Achromotrichia, nutritional 107
Acid production by cells and tissues 137
Adrenal atrophy, compensatory 21
Adrenal cortex hormones 119
Adrenal cortical extract, assay of 74
Adrenal cortical hormones and carbohydrate metabolism 131
Adrenal gland, hypophysectomy and 86
Adrenal glands, carbohydrate metabolism and low atmospheric pressure 48
Adrenal insufficiency, condition simulating 10
Adrenal medulla in resistance to cold 72
Adrenal, x-zone sensitivity to hormones 160
Adrenalectomy, dehydration after 3
Adrenalectomy during normal and deficient diets 69
Adrenalectomy, K poisoning after 94
Adrenals and muscle water 3
Adreno-cortical transplants and hemodilution 86
Alcohol and post-pituitary antidiuresis 166
Alcoholic fermentation 136
Alkali ingestion in man, continued 110
Amebacidal action of ya-tan-tzu 146
Amine, propylmethyl, pharmacology of 143
Amines, central stimulation with 165
Amino acid deficiency 97
Amino acid deficiency in man 116
Amino acid synthesis in chick embryo 107
Amino acids and growth 100
Amino acids, reaction of formaldehyde with 111
Aminobenzoic acid, achromotrichial action of 58
Aminobenzoic acid and hormones 98
Aminobenzoic acid inhibition of sulfonamide 78
Aminobenzoic acid, para-, toxicity of 71
Amino composition of animal tissues 187
Amyloid tissue, polysaccharide from 178
Androgen pellets, intramesenteric 12
Androgens, excretion in Addison's disease 128
Androgens, urinary, determination of 131
Androsterone, 48-hour response in rat 33
Anemia, iron retention in 26
Anemia, x-ray, and cobalt 30
Anesthesia, pentabarbital, duration of 163
Anesthesia, pentothal, and posture 80
Anesthetic potency and physical properties 147
Anesthetics and balance between chemoreceptor and nervous control 150
Anesthetics and toxic gases, safety margin of 157
Anesthetics, local, convulsive action of 144
Anesthetics, local, polarographic study of 160
Angiotinin destruction by tissues 114
Aniline produced methemoglobin 146
Antibodies, relation to immunity and infection 176
Antibody measurement 180
Arsenic analogue of choline 156
Anticataractogenic effect of diamino acids 188
Arsenicals, toxicity of 154
Ascorbic acid, antioxygenic action of 104
Ascorbic acid requirements of children 191
Autonomic centers after convulsions 3
Autonomic drugs and contractions of colon 171
Axon, ionic impedance of 15

B

Ballistic studies on hydraulic models 21
Barbiturates, cross tolerance to 13
Barbiturates, tolerance and cross tolerance to 152
Barbiturates, toxicity of 13
Barbituric acid, toxicity of 143
Barometric pressure, effect of lowered, on man 29
Benzedrine and O_2 consumption after morphine 153
Benzoate oxidation by mycobacteria 101
Bile acid synthesis 118
Bile pigment output on high fat diet 25
Bile salts and action of acetylcholine 138
Biotin deficiencies 191
Blacktongue, canine, water balance in 189
Blood after ligation of hepatic ducts 129
Blood amino acids, determination of 139
Blood, anticoagulants and specific gravity of 52
Blood, arterial lesions, diet and renal insufficiency 179
Blood capillaries, caliber changes of 14
Blood capillary fragility and bleeding time 44
Blood changes following sugar injections 30
Blood count and cyclotron radiations 180
Blood, determination of thiamin in 110
Blood, fetal, gaseous content of 82
Blood flow, apparatus for measuring peripheral 91
Blood flow following venous occlusion 68
Blood flow, heated thermocouple for 144
Blood flow in hemorrhagic shock 32
Blood flow, intestinal, and intestinal contractions 49
Blood flow measurement with thermostromuhr 34
Blood flow, renal, in hypertension 17
Blood flow, peripheral, in cardiac cycle 155

Blood flow, vasoconstriction and viscosity 65
Blood hemoglobin solutions, preservation of 43
Blood in exercise, acid-base equilibrium of 86
Blood iodine, placental transmission of 148
Blood lipids after pancreatectomy 2
Blood, osmotic pressure of 48
Blood plasma and "available fluid" volumes 126
Blood plasma, antithromboplastic activity of 85
Blood plasma, "poising" of 106
Blood pressure 74
Blood pressure and activated sterols 61
Blood pressure and pulse rate of newborn 39
Blood pressure and respiration in strychnine convulsions 141
Blood pressure of eskimo 121
Blood pressure, play of vasomotors and 75
Blood pressure, pressoreceptors and 16
Blood pressure raising principle in thermal shock 64
Blood pressure, venous, and cord lesions 92
Blood protein regeneration 106
Blood serum albumin, crystalline, denaturation of 127
Blood serum bilirubin 12
Blood serum iodine fractions 123
Blood serum phosphatase in protein-deficiency 42
Blood stream, disappearance of dye T-1824 47
Blood substitute, hemoglobin solutions as 3
Blood sugar and liver glycogen after "sulpha" drugs 34
Blood, toluene level of 149
Blood transfusion, incompatibility and donor's diet 59
Blood vessel permeability and lemon citrin 169
Blood vessel response to intrapulmonary pressure 39
Blood vessel tone 56
Blood vessels of finger, reactions to cold 75
Blood vessels of retrolingual membrane of frog 52
Blood, ultraviolet radiation of 61
Blood volume in newborn 20
Bone marrow absorption of purgative oils 53
Bone marrow, chemistry of 124
Bone marrow, Pasteur effect in 89
Bone studied by diffraction technique 70
Brain, action potentials of cerebellum 22
Brain, activity of geniculostriate system 57
Brain, activity of spinal-cerebellar tract 35
Brain, auditory sensibility representation in cerebellum 84
Brain brei oxidations, hemins and 134
Brain cephalin 110
Brain, cerebellar ablation and contractions of antagonistic muscles 60
Brain, cerebellar stimulation after decerebration 56
Brain, chronic state of decerebration 55
Brain, conductance and cellular permeability of 82
Brain conduction in convulsions 41
Brain cortex, acoustic area of 1
Brain cortex, irradiation in 2
Brain cortical commissures and visual perception 81
Brain cortical foci for gastrointestinal tract 64
Brain depressant drugs, action under nembutal 147
Brain, electrical activity of 29
Brain, frequency-localization in auditory cortex 51
Brain lesions and anesthesia, effect on vestibular nystagmus 60
Brain, localization in visual cortex 84
Brain, metabolism of fetal 69
Brain oxidations, serum and 96
Brain oxygen concentrations 19
Brain, response of auditory cortex with cochlear lesions 88
Brain, reticulocytosis as sign of frontal damage 61
Brain, salivatory "points" in lower stem 88
Brain, tactile sensibility representation in cerebellum 82
Brain, temperature and O_2 consumption 27
Brain tissue, oxidations in 108
Brain tissue, respiration and glycolysis of 58
Bromide distribution and excretion 101
"Butylnitroso" for trace metals 99

C

Cantherium, plasmodium, infection of canaries 144
Carbohydrate formation after feeding histidine 103
Carbon, heavy, as tracer element 115
Carbon monoxide, determination of 35
Carbon monoxide poisoning 170
Carbonic anhydrase 133
Carcinoma, adrenocortical, a new triol from 115
Carcinoma, intraocular transplantation 183
Carcinoma, temperature on growth and metastasis 181
Cardiac glycosides and lactones 14
Carotid body stimulation by gases 93
Carotine oxidase, inhibition of 99
Casein hydrolysis, first stages of 117
Casein, oxalic acid hydrolysate of 112
Casein, oxy-, nutritional assay of 100
Catalase activity, measurement of 140
Cerebrospinal fluid and serum, distribution of bromide in 90
Cerebrospinal fluid, concentration of 118
Chagas' disease, chemotherapeutics of 160
Cholesterol of blood serum 135
Cholic acid, bacterial oxidation of 133
Cholic acid production by rat 139
Choline deficiency 189
Choline esterase, ether and chloroform 169
Cholinesterase inhibition by products of physostigmine 150
Chymotrypsin, specificity of 112
Circulation and lung movement 9
Circulation, collateral, after arterial occlusion 33
Circulation, collateral, and vascular reactions 32
Circulation, recoil curve of 36
Cobra venom and thiamin, synergism of 54
Cochlea, frequency map of 18
Colchicine-like compounds, mitotic activity of 175
Colon, segmental activity of 1
Color analysis, photoelectric 116
Colorimetric standards 127
Complement fixing antibodies 183
Complement nitrogen, fixation of 178
Complement, ultracentrifugal properties of 176
Corticoadrenal extract 10
Corticotropin assay 4
Creatine excretion in E deficiency 190
Creatinuria after caffeine 4
Cresylic acids, toxicity and antiseptic action 146
Curare-like action of new synthetic agents 157
Curare-like agents, anticonvulsant action of 157
Cyclopropane and chloroform on central origins of sympathetic system 159
Cyclopropyl vinyl ether 156
Cystein and radio-active sulphides, sulphur exchange 134
Cystine and cysteine estimation 137
Cystinuria 115

D

Deafness "neurosis" 23
Demoral, addiction liability of 153
Demoral, effectiveness as analgesic 143
Demoral, properties of drug 15
Demoral, tolerance to 142
Desoxycorticosterone and electrolyte-water exchange 114
Desoxycorticosterone in uremia 73
Diabetes, disposition of glucose in 128
Diabetics, glucose from protein in 22
Diet and epithelial hyperplasia in forestomach 192
Diet and thiamine requirement 87
Diet, self-selection 89
Digitalis assay on embryonic chick heart 157
Digitalis bio-assay in man 151
Digitalis glycosides and cardiac hemorrhages 170
Digitalis, standardization of 154
Dihydrotachysterol, bioassay of 52
Dilantin derivatives, anticonvulsant properties of 156
Discrimination of color and form 48
Diuresis and water balance 1
Diuresis with progesterone and desoxycorticosterone 94
Diuretics, bioassay of 158
Diuretics, mercurial, local action of 144
Duodenum, activity during nausea 43
Dust inhalation and pneumonia 5

E

Ear, cochlear responses 51
Encephalogram after hypoglycemia and electroshock 28
Encephalogram, effect of K, Ca and Mg on 76
Encephalogram, electric 19
Encephalogram under low barometric pressure 46
Encephalograms in Jacksonian seizures 65
Energy exchange, work and body size 73
Energy expenditure in swimming 44
Enzyme conversion of quinine by tissues 97
Enzyme conversion of quinine in vivo 97
Epinephrine, absorption through bone marrow 53
Epinephrine toxicity 71
Ergot alkaloids, determination of 167
Erythroblastosis fetalis, Rh factor in 180
Erythrocyte fragility during lipemia 51
Erythrocyte mass and venous hematocrit 177
Erythrocyte volume with radioactive tagged cells 183
Erythrocytes, disk-sphere transformation of 68
Estrogen excretion 91
Estrogens and phenolases 31
Estrogens, conjugated, in pregnancy urine 69
Estrone and estradiol, colorimetric method for 169
Ethers, biochemorphology of 159
Excretion of thiamin 111
Exercise, cardiovascular response to 39
Exercise, effect of, on circulation 23
Extracellular fluid, bromide determination of 111
Extracellular fluid volume in man 31
Eye, galactose cataract, minerals and 132
Eye, penetration of sugars in aqueous humor 90
Eye, rod and cone dark adaptation 79

F

Fatigue, double work periods in study of 25
Fats, glyceride structure of 122
Fatty acids, halogenated 161
Fatty aldehydes, higher 139
Fibroma-myxoma transformation 183
Flow of fluid in elastic system, dynamics of 43

G

Galvanized nails, "mouthing," an industrial hazard 168
Gastric and duodenal motility 66
Gastric antacids and gastric secretions 141
Gastric emptying and acclimatization to altitudes 83
Gastric evacuation, hydrochloric acid and 68
Gastric juice, histamine content of 15
Gastric juice, rennin of 21
Gastric pouches, vagal and non-vagal 118
Gastric secretion, effect of insulin on 62
Gastric secretion, effect of urogastrone on 78
Gastric secretion, influence of bile on 45
Gastric tissue resistance and electrical activity 70
Gastro-intestinal absorption of lanatoside C 149
Glucose of depancreatized dog 106
Glucose phosphorylation in erythrocytes 107
Glycine and zinc compounds 113
Glycosides, cardiac, potency in spinal cat 161
Glycosides, emetic responses of 22
Gold salts, tolerance to 148
Gonadotropic effect of steroids 2
Gonadotropic hormone, differentiation of 102
Gonadotropic hormone, preparation of 119
Gonadotropins, ovarian response to 101
Growth, aniline and *p*-aminodimethylaniline 140
Guinea pig, dietary essentials of 193

H

H production in green algae 112
Heart, action of cyclopropane on 64
Heart, action of pituitary extract on 77
Heart, action of unsaturated lactones on 148
Heart, Ca and K reversal effects on 17
Heart, chemical changes in hypertrophy 116
Heart, conditioned reflexes to painful stimuli 28
Heart, coronary flow during pulmonary stenosis 34
Heart, coronary occlusion and fibrillation 37
Heart, digitalis and cardiac output 150
Heart, effects of high O_2 pressure 92
Heart, fractionate contractions and potentials of 24
Heart, fractionate contractions on surface of 29
Heart, hypertrophy in relation to cholesterol 26
Heart, intra-thoracic pressure and cardiac output 42
Heart muscle contraction and xanthines 156
Heart muscle, phosphate exchange in 27
Heart muscle, potentials and tonus of 9
Heart, nature of S wave 62
Heart, potentials distribution in leads I and III 45
Heart, quinidine and ventricular fibrillation 90
Heart, ventricular fibrillation, galvanic thresholds for 35
Heart-lung preparation, high serum K and 95
Hemolysis by potassium salts 19
Hemorrhagic disease, inherited, of swine 125
Hemorrage, renal humoral mechanism in 16
Heparin, an antidote to trypsin 149
Heparin and toxicity of ouabain and digitalis 53
Heparin effect and method of administration 54
Heparin, physical agents and stability of 54
Hepatomas, spontaneous primary 175
Heroin, urinary excretion of 127
Hippuric acid synthesis 97
Histamine antagonists in gastric secretion 9
Histamine release in allergic skin 154
Hormone, lactogenic, action on tissues 33
Hormones of hypophysis and organic solvents 170
Hydrogen ions, measurement of 115
Hydroxyethylapocupreine, action of 162
Hyperemia, reactive 20
Hypertension and intracisternal kaolin 47
Hypertension, experimental, and kidney extracts 34
Hypertension, renal, treatment with renin 88

I

Industrial solvents, toxicity of 160
Insulin action in muscle *in vitro* 50
Insulin and pyruvate formation 12
Insulin, hypoglycemia after 23
Insulin test of vagal supply to stomach 116
Insulin tolerance after hypophysectomy 11, 164
Intestinal obstruction, azotemia and 40
Intestinal synthesis of biotin 134
Intestine, allergic inflammation of 177
Intestine, secretion of phosphatase 47
Iodine excretion by salivary and gastric glands 109
Iso quinoline derivatives, antispasmodic action of 148

K

Kerosenes, comparative toxicity of 151
Ketone bodies, utilization of 135
Ketosteroid, crystalline conjugated 139
Kidney anti-pressor extracts, assay of 114
Kidney, deamination of alanin by 13
Kidney excretion of electrolytes in Addison's disease 130
Kidney excretion of nicotine 152
Kidney, exteriorized, circulation in 179
Kidney, injection of drugs via ureter 158
Kidney insufficiency and K clearance 180
Kidney, normal, secretion of renin by 10
Kidney production of NH_3 in acidosis 67
Kidneys, explanted, circulatory alterations in 173

L

L. casei ϵ eluate factor in nutrition 190
Lactation after adrenalectomy 28
Lactic acid, distribution in blood and eye 38
Lactoglobulin, β-, molecular weight of 103
Larvicidal action of chloro-hexyl-meta-cresol 146
Lecithin intravenously 185
Lipid content of endometrium 59
Lipids of fasting mouse 123
Lipids of tubercle bacilli 135
Lipocaic and inositol after pancreatectomy 65
Lipocaic and insulin sensitivity 22
Liver arginase 139
Liver blood flow and glucose output 18
Liver cirrhosis, dietary, prevention of 187
Liver detoxification of pregnanediol 49
Liver fat and diet after pancreatectomy 76
Liver fat, brominated fatty acids and 99
Liver fat, effect of inositol on 124
Liver, hemorrhagic necrosis and cirrhosis 188
Liver in alimentary azotemia 36
Liver in experimental uremia 8
Liver in metabolism of estradiol 79
Liver, O_2 consumption with monopiperidine 162
Liver response to insulin and prolactin 72
Liver vitamin A, phenolphthalein and sulfanilamide 168
Low O_2 and respiratory response to CO_2 167
Lymphogranuloma, chemotherapy and 182

M

Magnesium, colorimetric determination of 130
Magnesium, metallic, tissue response to 186
Mammary gland hyperplasia 186
Manometer, glass membrane optical 32
Measles virus, egg-passed 184
Membrane, response to tuning fork vibration 6
Membrane semipermeability 171
Menstruation, basal functions and 80
Metabolism, basal, cobra venom and opiates on 53
Metabolism, basal, in acne vulgaris 5
Metabolism, basal, previous diet and 132
Metabolism, carbohydrate 121
Metabolism, carbohydrate, after evisceration 76
Metabolism, energy, effect of insulin and thyroxin on 77
Metabolism in anorexia nervosa 192
Metabolism, iodine, in thyroid gland 123
Metabolism, ketone, in mouse 120
Metabolism, level of dietary protein and 108
Metabolism of acetic acid 137
Metabolism of alanine, intermediary 114
Metabolism of bacteria exposed to sulfonamide 46
Metabolism of benzyl chloride 136
Metabolism of dilantin derivatives 153
Metabolism of ethyl alcohol, pyruvate and 140
Metabolism of fructose 5
Metabolism of glucose by bacteria, riboflavin and 120
Metabolism of N-derivatives of amino acids 103
Metabolism of nicotinic acid and sulfapyridine 108
Metabolism of pantothenic acid by bacterium 108
Metabolism of paramecium, phosphorus and 59
Metabolism of progesterone 41
Metabolism of pyruvic acid 111
Metabolism of radioactive iron 175
Metabolism of recinoleic acid 137
Metabolism of testosterone and estradiol 155
Metabolism of thiamin and riboflavin 137
Metabolism, protein, and pituitary growth hormone 65
Metabolism, protein and purine 99
Metabolism, phosphorus, of nuclei 57
Methionine, α-keto from 104
Methionine, estimation of 120
Metrazol detoxification, site of 25
Micturition volume in rat 50
Milk, humoral control of let down of 66
Mineral deficiency 106
Morphine addiction and serum cholinesterase 171
Morphine analgesia and prostigmin 167
Morphine poisoning, coenzyme of liver and muscle 169
Morphine poisoning, O_2 uptake of brain in 166
Morphine poisoning, O_2 uptake of muscle in 166
Morphine, pyretic effects of 153
Muscle activity and intergration of action potentials 82
Muscle and nerve regeneration 85
Muscle, diaphragm, fibrillation in 95
Muscle, distribution of radioactive P in 164
Muscle efficiency, ouabain and 146
Muscle electrograms of volitional twitches 86
Muscle, exchange of K for NH_3 in 24
Muscle fatigue 70
Muscle fiber, chloride content of isolated 38
Muscle fiber, method to record dimensions of striae 38
Muscle fiber, unit of fibrillary activity 38
Muscle fibers, single, action potentials of 29, 31
Muscle, glycogen metabolism of 49

Muscle, intestine, sensitization to adrenalin ... 96
Muscle, isometric contractions of ... 77
Muscle myosin accumulation of K ... 61
Muscle myokinase and adenylpyrophosphatase ... 119
Muscle, Na associated with extracellular acid ... 93
Muscle proteins from rat and octopus ... 125
Muscle, radio-P and P-compounds in ... 109
Muscle respiration, iodoacetate and iodoacetamide on ... 83
Muscle, smooth, phenyl-piperidine derivatives and ... 151
Muscle, training pattern of ... 56
Muscle, uterine, viburnum prunifolium and ... 151
Muscle work capacity and ergotamine ... 163
Mucosa, rectal, concentration and absorption by ... 83

N

Neoplasms, human, in tissue culture ... 176
Neoplasms, malignant, lymphocyte response in ... 185
Neosynephrine and amphetamine isomers on coronary circulation ... 151
Nerve blocking after thyroparathyroidectomy ... 24
Nerve fibers, single and acoustic stimulation ... 27
Nerve, pathways in heat regulation ... 7
Nerve, potential and cholinesterase activity in ... 62
Neurohumoral mechanism in gradation of stimulation ... 29
Neurohumoral nervous intergration ... 10
Neuromuscular states under certain drugs ... 43
Neuromyal junction ... 6
Neurons, spinal, excitation of ... 71
Neurophysiology, roles of cH in ... 29
Nicotine and physostigmine, antagonism between ... 164
Nicotine, fate of, in dog ... 49
Nucleic acid of tobacco virus ... 105
Nutrition, requirements of hamster ... 191

O

O_2 consumption of yeast and cell division ... 24
O_2, low, acclimatization to ... 87, 167
O_2, low, iodoacetate and survival ... 40
Obesity, experimental production of ... 11
Obesity, hypothalamic ... 10
Ortho-nitro-phenol, pharmacology of ... 145
Ova, fertilized, after first menstruation ... 178
Ovulation, induced ... 73
Ovum, nutrition of ... 67
Oxidation, pyruvate, acetyl phosphate in ... 122
Oxygen poisoning ... 8
Oxygen-reduction potential requirements ... 114

P

P, fecal, endogenous and exogenous ... 98
P, labeled, distribution and excretion ... 104
Pain mechanisms in stomach ... 95
Pancreatectomy after hypophysectomy ... 46
Pancreatic diabetes in monkey ... 31
Pancreatic diabetes in the owl ... 61
Pancreatic secretion, drugs stimulating ... 72
Pantothenic acid deficiency ... 133
Pantothenic acid excretion and diet ... 191
Papain activating power of blood ... 152
Paraldehyde, tolerance to ... 13
Paraldehyde, toxicity of ... 45, 67
Pentachlorophenol, toxicity of ... 148
Phage, neutralized, reactivation of ... 179
Pharmacology, new experimental methods in ... 161
Phosphatase, intestinal, intravenous injection of ... 181
Phosphate, calcium, pH and solubility of ... 113
Phosphorus, radioactive, distribution in leukemia ... 186
Pitressin NNR, reaction of tubes and ovaries ... 151
Pituitary, action of purified protein from ... 105
Pituitary preparations and cardiovascular effects ... 172
Placenta, passage of radioactive iron ... 182
Placenta, transfer of water across ... 28
Planta cundeamor ... 164
Pneumocardiogram ... 7
Pneumococcic antibody nitrogen, determination of ... 174
Pneumococcus, heterophile antigen of ... 112
Poliomyelitis from "neurotropic" streptococci ... 182
Polycythemia, experimental ... 19
Polysaccharide produced by crown-gall ... 129
Porphyrinuria, a sign of lead poisoning ... 132
Postural stability and body alignment ... 39
Pregnancy and blood pressure in hypertension ... 176
Pregnancy urine, ketonic steroids of ... 66
Pregnancy, vascular responses in ... 1
Pregnancy, water and electrolyte distribution ... 105
Protein-antiprotein reactions in sera ... 178
Protein, deficient, oxidation of casein into ... 138
Protein intake and excretion of vitamins ... 132
Proteinase of wheat gluten ... 128
Proteins, catalysis of hydrolysis in ... 136
Proteins, extraction from aqueous solution ... 98
Proteins, precipitation of ... 125
Proteins, reaction of carbon suboxide on ... 131
Proteins, synthetic detergents and ... 126
Pulse wave, propagation of ... 20

Q

Quinine, photelmetric estimation of ... 120

R

Radiation of sarcoma and abnormal mitosis ... 177
Radiation, ultraviolet, and body weight ... 8
Radioactive Ca and Sr, biological effects of ... 163
Radioactive iron, absorption of ... 174
Reflexes, conditioned ... 30
Reflexes, conditioned, metrazol convulsions and ... 74
Reflexes, long spinal, activity pathways in ... 51
Renal humoral mechanism in shock ... 35
Renal pressor system ... 17
Renin response and tachyphylaxis in hypertension ... 63
Renin, specificity of ... 6
Resins for fruit coatings, hazards of ... 168
Resorcinols, anthelmintic action of ... 142
Respiration affected by glycogen and biotin preparations ... 122
Respiration, control of, by carotid and aortic body ... 90
Respiration, effect on left and right ventricles ... 80
Respiration of spinal cord after asphyxiation ... 87
Respiration of spinal ganglia ... 85
Respiration, single fiber analysis of inhibition of ... 40
Respiratory effects upon visual threshold ... 88
Respiratory exchange and food habits ... 84
Respiratory modifiers after brain stem transection ... 42
Respiratory phrenic neuroses ... 67
Respiratory effects of brain stem transections ... 63
Respiratory reflexes from limbs ... 16
Resuscitation of newborn, O_2 and CO_2 in ... 7
Riboflavin deficiency, induced ... 192
Ribonuclease and thymonucleo-depolymerase ... 113

S

Salivary gland tumors, induced ... 185
Salt and water ingestion ... 17
Secretin, peptogogic effect of ... 11
Semen, prostatic and vesicular fluid, composition of ... 79
Serine, colorimetric determination of ... 102
Shock, after sympathectomy and cord transection ... 78
Shock and electrolytes ... 57
Shock, desoxycorticosterone in ... 44
Shock, gravity ... 59
Shock, hemorrhagic ... 91, 92
Shock, traumatic, toxic factor in ... 81
Sperm ejaculation by uterine drug ... 143
Sperm, survival of ... 75
Spinal cord effect of tetanus toxin ... 4
Sporotrichosis, chronic progressive, in mice ... 173
Steroid compounds, anesthetic action of ... 79
Steroids, effect on vaginal mucosa ... 14
Steroids in Addison's disease ... 26
Sulfadiazine absorption from peritoneal cavity ... 141, 142
Sulfanilamide and phytotoxic reactions of blood ... 55
Sulfanilamide, antagonists of ... 47
Sulfanilamide, blood levels of ... 173
Sulfanilamide chanosis ... 164
Sulfanilamide, propylene glycol solutions of ... 172
Sulfonamides, propylene glycol solvent for ... 172
Sulfapyradine toxicity in vitamin B deficiency ... 147
Sulfapyrazine ... 131
Sulfathiazole, succinyl, inhibitor of bacterial syntheses ... 171
Sulfathiazole, succinyl, toxicology of ... 159
Sulfides, pharmacology of ... 155
Sulfonamide, acetyl, deacetylation of ... 165
Sulfur, radioactive ... 138
Sulphonamides in peptone fever ... 145
Sweat concentration after desoxycorticosterone ... 41
Sympathetic ganglion inhibition ... 57

T

Taste, hyperphagia and adiposity ... 76
Taurine formation from cystine ... 125
Temperature and response to anoxia ... 126
Theophylline, toxicity of ... 162
Thermostromuhr method, errors in ... 80
Thiamine in urine, estimation of ... 123
Thiamin, riboflavin and nicotinic acid deficiency ... 117
Thiamine deficiency, polyneuropathy in ... 189
Thrombin, purification of ... 184
Thromboplastic properties of digitaloid drugs ... 55
Thromboplastin, comparison with viper venom ... 130
Thyroid, activity of goitrous ... 50
Thyroid, effect of "sulpha" drugs on ... 122
Thyroid function and environmental temperature ... 72
Thyroidectomy ... 24
Thyroidectomy, action of estrogenic hormone after ... 63
Thyroidism, hyper-, weight of heart and adrenals in ... 62
Thyrotropic hormone assay ... 18
Tissue assimilation of radioactive carbon ... 109
Tissue respiratory ferment in chick embryo ... 129
Tobacco smoke, respiration and blood pressure ... 44
Tomato, nutritive value of ... 189
Tongue, pseudomotor phenomenon of ... 93
Toxicity of blue-green algae ... 124
Toxicity of certain alkaloids ... 37
Toxicity of diamidino stillbene and tolamidine ... 167
Toxicity of pontocaine ... 89
Tryptophane and urinary output of acetone ... 102
Tryptophane deficiency, pathological changes in ... 175

Tuberculin and behavior of rats and fishes..... 54
Tuberculin, reactivity to..... 182
Tuberculosis, efficacy of drugs in..... 181
Tuberculosis, methyl-isopropyl-phenol in..... 145
Tungstate, sodium, subcutaneous injection of..... 165
Tyrosine, determination of, in blood filtrates..... 101

U

Ulcers, permeability in etiology of..... 22
Urea, chloride and water retention..... 94
Urethane methiodides, eserine-like action of..... 154
Urogastrone and enterogastrone..... 37
Uronic acids, colorimetric determination of..... 125
Uterus, consequences of asphyxiation in..... 93
Uterus, response to sympathomimetic drugs..... 164

V

Vaccinia infection and edema..... 184
Van den Bergh reaction..... 106
Veratridine action on heart-lung preparation..... 161
Veratrum alkaloids and circulatory reflexes..... 156
Vitamin A, carotene and xanthrophyll..... 105
Vitamin A deficiency and ciliary movement..... 174
Vitamin A depletion..... 188
Vitamin A intake, relation to growth..... 121
Vitamin A utilization..... 134
Vitamin A, ultraviolet absorption curve of..... 126
Vitamin B and utilization of galactose and glycine..... 110
Vitamin B complex of yeast, utilization of..... 129
Vitamin B deficiency benefitted by thiamine..... 174
Vitamin B, effect of, on muscular and mental work..... 81
Vitamin C..... 7
Vitamin C excretion..... 100
Vitamin content of rice polishings..... 190
Vitamin E and muscle degeneration..... 117
Vitamin K anemia..... 177
Vitamins in preventing neuron degeneration..... 186

Z

Zinc deficiency..... 188

CORRECTIONS OF ERRORS IN MARCH ISSUE

Page 106. Cohn and Greenberg. Correct title to read: "The biochemistry of magnesium deficiency. III."

Page 113. Greenwald. Correct last two formula lines in abstract to read:

$$[Zn(^{+}H_3NCH_2COO^{-})_3 \begin{matrix} O^{-} \\ OH \end{matrix}] Na \cdot H_2O$$

$$[Zn(^{+}H_3NCH_2COO^{-})_4 \begin{matrix} O^{-} \\ O^{-} \end{matrix}] Na_2 H_2O$$

Page 136. Stern. Correct sentence beginning on line 17 of abstract to read: "Pyrophosphate and α,α'-dipyridyl, which likewise form complexes with ferrous iron, cause a marked inhibition. Azide, which reacts with ferric iron, and 8-hydroxyquinoline, thiourea, and salicylaldoxime, which have a high affinity for copper ions, are ineffective even in high concentrations."

Page 158. Lipschitz and Hadidian. From line 24 to end of paragraph of abstract correct to read: ". . . case of the xanthine diuretics and salyrgan. The activity of urea was taken equal to unity and all other activities referred to this. The mean excretion in more than 100 control groups was 32 per cent of the fluid administered; while with high doses of some diuretics over 100 per cent was obtained. The following is a comparison of the relative diuretic activities found by the rat method with the relative activities (figures in parenthesis) calculated from human therapeutic doses: urea, 1 (1); sodium acetate, 2.7; ammonium chloride 2.8 (2), potassium acetate, 3, (2.1); sodium nitrate, 4.9; potassium nitrate, 5 (4); theobromine, 12.6 (150); caffein, 72.5, (625); theophylline, 140, (480); salyrgan 700, (1250); biuret, 2.2."

Page 160. McIntyre and King. Correct first two words of abstract to read: "Polarographic investigation."

Page 192. Small and Milhorat. In fourth line of second column of abstract correct "were determined" to read "was determined."

NOTES ON THE BOSTON MEETING, March 31, April 1, 2, 3, 4, 1942

The Federation met under the Chairmanship of Dr. Arthur G. Hogan, President of the Institute of Nutrition. The Joint Session was held Thursday forenoon with a program, arranged by Dr. Arthur H. Smith, Secretary of the Institute, consisting of one speaker from each of the five constituent societies as selected by the several councils. The scientific sessions began Wednesday morning and continued until Saturday noon. The meetings of each society were assigned to different hotels. For the first time the abstracts of all the scientific papers presented were published in the Federation Proceedings.

Although the Nation's preparations for war were already mounting the attendance was larger than the Local Committee anticipated. There was a registration of 2083, 751 members and 1332 non-members, each paying a registration fee of $1.00. No Annual Dinner was held; instead the Local Committee provided a Smoker Thursday evening with refreshments, entertainment and music for dancing for which a charge of fifty cents was made and 1296 tickets were sold. The Local Committee received $2751.00 and spent $1401.51, leaving a surplus of $1349.49 for the Federation Treasury. One thousand and forty-five copies of the Federation Program were sold at 50¢ each and six hundred copies of the Federation Proceedings were sold at $1.50 each.

Significant actions of the Executive Committee, as approved by all the constituent societies, were:

1. Extension of an invitation to the American Association of Immunologists to become a constituent member of the Federation. This invitation was later accepted.

2. Decision to meet in Cleveland in 1943 and in New York City in 1944.

3. To assess each constituent society five cents per member for the expenses of the Committee for the Protection of Biological Research and one dollar per member to finance the Federation Proceedings and the Federation Office.

4. To hold a joint session at Cleveland arranged by the secretaries of the societies and to omit the Federation Dinner.

5. To designate Dr. H. B. Lewis Director of the Placement Service.

6. To reappoint all standing committees as now constituted except the Cleveland Committee on Public Information, the latter to be: Harry Goldblatt, Chairman, R. G. Hoskins and R. W. Gerard.

7. To designate Dr. D. R. Hooker Secretary-Treasurer.

8. To effect a revision and extension of the By-Laws to accord with the plan of publication of the Federation Proceedings.

9. To leave the style of abstracts to the individual societies.

10. To have the Federation Secretary set the times of the Business Meetings of the constituent societies so that business may more readily be referred from the Executive Committee to the societies and from the latter to the former.

B & Z Library

Papers Presented before the Joint Session of the Federation

Boston, April 2, 1942

METABOLISM OF THE ESTROGENS

EDWARD A. DOISY, S. A. THAYER AND J. T. VAN BRUGGEN

Laboratory of Biological Chemistry, Saint Louis University School of Medicine, Saint Louis

Since the first crystalline estrogen was isolated only about thirteen years ago (8, 18) and the natural compounds are still expensive and difficult to obtain, it is not surprising that their metabolism is still largely a matter of doubt and speculation, and that only a few experiments designed for the study of their metabolism have appeared in the literature. Consequently, it will be necessary and, of course, under the circumstances desirable, to gather information which has a bearing on their metabolism from all other types of investigation. Perhaps the taking of stock may be of value in stimulating additional investigations on the anabolism and catabolism of estrogens.

A. *Anabolism of Estrogens.* The restoration of certain phases of estrus by the administration of estrogens to ovariectomized rodents (Allen, 2) in which those phenomena do not occur following the removal of the ovaries leaves little doubt that at least the final stage of estrogen production occurs in the ovary. Other tissues may also produce estrogens but the evidence is not so definite as for the ovary.

The indispensability for life of the adrenal cortex seems to depend upon the capacity of that gland to synthesize steroid hormones. In addition to the compounds which may be regarded as cortical hormones, an androgen, adrenosterone (Reichstein, 62), and an estrogen, estrone (Beall, 4), have been isolated and identified. Perhaps these compounds were not synthesized in the adrenal but the following evidence lends some support to the view that the adrenal can produce sex hormones.

(1) Feminizing effects and excretion of abnormal amounts of estrogens observed in males having certain types of tumors of the adrenal and the loss of the feminization following successful removal of the tumor have been reported (Simpson and Joll, 67; Holl, 40).

(2) Nodular hyperplasia of adrenals, development of mammary glands and estrous cycles including uterine growth in dba strain mice which had been ovariectomized at birth (Woolley, Fekete, and Little, 81).

(3) Administration of extracts of the pituitary which produce stimulation of the adrenal cortex to young ovariectomized rats caused establishment of the vaginal introitus (Moon, 56) and enlargement of the sex accessories (Nelson, 57).

(4) The greatly delayed opening of the vagina or its complete absence in ovariectomized-adrenalectomized rats (Wade, 74).

(5) The excretion of estrogens by ovariectomized women (Frank, 25).

Certain evidence indicates but falls short of conclusive proof that in the pregnant state the placenta secretes estrogen.

(1) Three crystalline estrogens have been isolated from human placenta (Browne, 7; Westerfeld, et al, 79; Huffman, et al, 42).

(2) During pregnancy the excretion of estrogens is much greater than during the non-pregnant condition. Since the excretion diminishes very rapidly following delivery, it seems likely that the placenta has produced the estrogens.

(3) Removal of the ovaries of pregnant women (von Probstner, 61; Guldberg, 32; Allan and Dodds, 1; and others) or mares (Hart and Cole, 34) did not cause a disappearance of estrogens from the urine. (Since the importance of hydrolysis of conjugated estrogens was not fully appreciated at the time these investigations were conducted, it is not surprising that many of the values are lower than those now generally reported for urine of pregnancy.)

Amount of estrogen produced by non-pregnant and pregnant women. The quantity of estrogen produced during a menstrual cycle can be approximated by two different procedures: 1, the amount of estrogen required to produce enough uterine growth in the castrate to permit subsequently administered progesterone to produce the typical premenstrual endometrium; 2, a study of the amount of estrogen which must be administered to an ovariectomized woman to produce an excretion of estrogen equivalent to that occurring during a normal menstrual cycle. Certain data in the literature are available for the application of both methods but it must be conceded that the agreement between results must not be given much weight and that the figures are of significance only in showing the *probable order of magnitude* of the monthly production of estrogen.

Corner (11) has pointed out that Willard Allen found that about 4,200 I.U. per day are required to build up the castrate's uterus sufficiently to produce "cessation bleeding." Werner (75) reported that the administration of 84,000 I.U. over a period of 21 days (i.e., 4,000 I.U. per day) and then sub-

sequent treatment with progesterone produced a premenstrual endometrium from which bleeding occurred following the cessation of treatment. These figures indicate that approximately 100,000 I.U. or 10 mg. of estrone are required to produce the full estrogenic reactions of the menstrual cycle.

For the other method of approximation very few data are available. Several investigators (Frank, 24; Koch, et al, 27; Smith, Smith and Pincus, 70; Gustavson, 33) have determined the amount of estrogen excreted during a normal menstrual cycle to be about one milligram. In their studies on the excretion following the administration of estrogens to two ovariectomized women, Kemp and Pederson-Bjergaard (46) found that about 10 per cent of the quantity administered was excreted. In one case 81,000 I.U. were administered and 10,000 I.U. appeared in the urine. Consequently, it seems likely from these data that the normal woman produces about 10 mg. of estrogen per menstrual cycle.

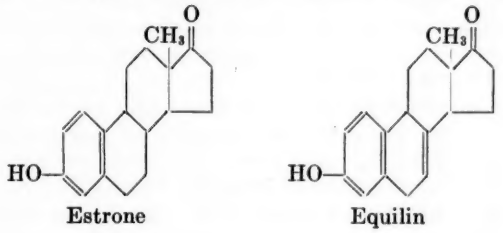
During the later stages of pregnancy about 30 mg. of total estrogens (Smith et al., 69; Bachman, 3) are excreted daily. If the velocity of the catabolic reactions proceeds at the same rate as in the non-pregnant state, approximately 150 to 600 mg. of estrogen must be produced during each twenty-four hour period. In view of the failure to find catabolic products of estrogens in the huge volumes of urine that have been extracted, it seems improbable that the organism produces such large quantities but, on the contrary, more likely that degradation is limited in the pregnant state.

Smith and associates (70) have reported that the presence of progesterone in the organism prevents the destruction of estrogens. During pregnancy the increased excretion of pregnanediol is generally regarded as an index of an increased production of progesterone. This may limit destruction, thereby allowing the excretion of a larger proportion of the estrogen.

Biological synthesis of estrogens. Though the synthesis of cholesterol by mammals is widely accepted, the intermediates used by the organism are still unknown.[1] The similarity of the ring structures of cholesterol and the estrogens and the degradation of cholesterol in vitro into some of the sex hormones has led to interesting speculations (Fieser, 21; Koch, 47; Marker, 50; and Marrian, 53) on the possible interconversions between the steroid compounds. As a stimulus to further research which may perhaps elucidate the transformation of some steroids to the estrogens these speculations are important but the fundamental problem which is still a mystery is the synthesis of the perhydrocyclopentenophenanthrene nucleus.

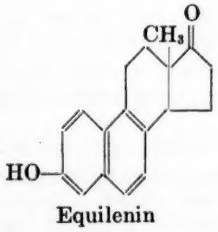
B. *Catabolism of Estrogens.* Since only a few publications contain experiments designed directly to study the catabolism of the estrogens, it will be necessary for us to glean as much information as possible from other sources. In our discussion, it must be borne in mind that all substances in the urine are not necessarily waste products or catabolites. For example, vitamin C, B_1, B_2, creatine, cholesterol, amino acids, and glucose certainly should not be regarded as catabolites but rather as products which have entered the urine in accordance with their concentration in the blood plasma and their specific relationship to kidney secretion. Consequently, the occurrence of an estrogen or a related product in the urine in itself is not a guarantee that the compound in question is a catabolite.

Metabolism of endogenous estrogens. The number and character of the estrogens isolated from the urine of pregnant mares affords an opportunity for speculation (29, 39, 44, 80). Since it appears that estrone is excreted in large quantities before appreciable amounts of the more highly unsaturated estrogens appear in the urine (28), it seems possible that the latter may be steps in the catabolism of the former. However, if we assume that estrogens are produced by partial degradation of a sterol the discovery of Δ-5,7,9-estratrienol-3-one-17 (Heard, 36) might be taken to suggest the reverse order in which the first attack on the cyclopentanophenanthrene nucleus is dehydrogenation in Ring B. Dehydrogenation of some saturated ring structures has been effected by mammals but the reduction of aromatic rings seems to occur less frequently.

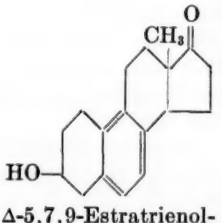


Estrone

Equilin



Equilenin

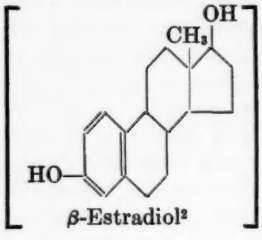


Δ-5,7,9-Estratrienol-3-one-17

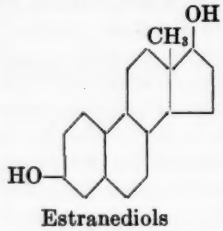
The products isolated from human urine give other clues to possible pathways of catabolism. α-Estradiol (41, 42), estrone (8, 18, 79) and estriol

[1] In a recent paper Bloch and Rittenberg (6) report that acetic acid is used in the synthesis of cholesterol.

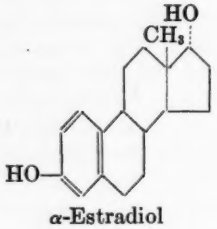
(7, 17, 52) have been isolated from human pregnancy urine as well as the human placenta. Per-



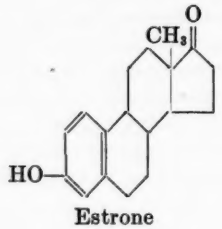
β-Estradiol[2]



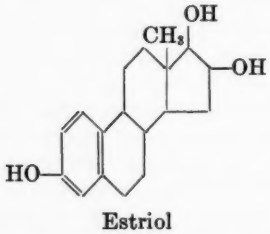
Estranediols



α-Estradiol



Estrone



Estriol

haps estriol is merely an oxidation product but since it has not been found in other than human sources, it may have a special but unknown significance in that species.

In rabbits the administration of both α-estradiol and estrone causes the excretion of β-estradiol (23, 35). Moreover, this compound has been found in the urine of pregnant mares (39). Although it was not found in the urine of the man who received 250 mg. of α-estradiol (37), a careful search should be made of human pregnancy urine for β-estradiol. If the failure thus far to find it is confirmed, the explanation may lie in species differences with respect to both β-estradiol and estriol.

Although Marker (51) concluded that the occurrence of estranediols which he obtained only from the urine of non-pregnant women indicates a difference in the metabolism of estrogens, the failure to find them in pregnancy urine does not constitute absolute proof of their absence. Their close similarity to estradiol indicates a catabolic or anabolic relationship to the estrogens. Since Heard and Hoffman (37) did not find them in their study of the metabolism of α-estradiol, it should be pointed out that estranediols may be steps in the production of estrogen.

The report of Dorfman (20) that he has found estrogenic potency which cannot be removed from an etheral solution by repeated (80 times) extraction with alkali leads one to wonder whether this non-phenolic product is a stage in the catabolism or anabolism of estrogens or perhaps is a product unrelated to the metabolism of the natural estrogens but yet possessing estrogenic properties. As far as the author knows Marker's estranediols and Heard's △-5,7,9-estratrienol-3-one-17 have not been tested for estrogenic properties; on the basis of their non-phenolic character both could appear in Dorfman's extract.

The conjugation of estrogens is another interesting point in their metabolism, i.e., estriol as the glucuronide (Marrian, 9) and estrone as the sulfate (Marrian, 64). In view of Marrian's report that virtually all of the estrogens of human pregnancy urine are in the conjugated form, it might be concluded that only those portions of the estrogens which are conjugated escape deep seated catabolic changes. A study of the metabolism of parenterally administered estrone-sulfate and estriol-glucuronide might yield interesting data on this point.

In addition to estranediols and estrogens known to occur in human urine, George and Olive Smith (71) have offered evidence that something other than estrone can be converted by hydrolysis and the reducing action of acid and zinc into a product having a higher estrogenic potency. This observation may indicate a new compound of importance in the catabolism of estrogens.

Metabolism of exogenous estrogens. After the discovery of the huge quantities of estrogen present in the urines of the pregnant woman and mare, it was something of a shock to find that the administration of estrogens led to the excretion of only a small percentage—5 to 20 per cent of that administered. This finding naturally led to an examination of the metabolism of estrogens. In this work, man, monkeys, horses, as well as the common laboratory animals, have been used. Needless to say, caution should be observed in the interpretation of the results obtained in one species from a consideration of data secured on a different species. Consequently, a brief resume will be given for each species which has been used in metabolism experiments.

Attack on the catabolism of estrogens has been conducted by conventional methods.

1. In vivo experiments. Examination of the excreta after the administration of a quantity of estrogen in excess of the capacity of the animal to effect complete oxidation.

[2] The formula for β-estradiol is enclosed in the bracket to indicate that this compound has not thus far been found in tissues or excretions of the human.

2. In vitro experiments. Examination of effects of extracts of various tissues.

Catabolism of estrogens by dogs. In one of the earliest experiments on metabolism, Carlson and his associates (48) injected 200 rat units daily for a period of 39 days. From the 39th day to the 42nd from 40 to 62 per cent of the estrogen in terms of rat units was found in the urine. In a recent series of experiments of shorter duration, Dingemanse and Tyslowitz (14) report the recovery of 10 to 50 per cent of estrogens in the urine and by assuming that the non-ketonic fraction was composed of the less potent estriol in one case in which estrone was administered, they calculate a recovery of 76 per cent in the urine. In a single experiment with estrone we have obtained data indicating a much greater destruction and the occurrence of estrogenic potency in the estriol, estrone and estradiol fractions without, however, obtaining positive identification of these compounds.

Using bile fistula dogs, Longwell and McKee (49) found that the estrogens of the bile after the administration of estrone were non-ketonic. Partition by Mather's (54) procedure indicated the presence of estrogens in both the estriol and estradiol fractions.

Catabolism of estrogens by rabbits, guinea pigs and rats. George and Olive Smith (68) reported that less than 10 per cent of estrogens administered to rabbits were recovered in the urine. However, injections of normal females with the gonadotropic hormone caused an increase of as much as 1,000 per cent; if the females had been ovariectomized the administration of the gonadotropic hormone had no effect but the injection of progestin caused the increased excretion of estrogen. Frank, Goldberger and Spielman (26) could not confirm the data reported by the Smiths but Pincus and Zahl (58, 60) not only confirmed them but added some additional points on the metabolism of estrogens by rabbits.

1. Estriol is not converted to estrone or estradiol.

2. "Estrone is converted into estriol in animals with a functional uterus.

3. "Estradiol is converted to estrone in animals with ovaries.

4. "Progesterone increases the amount of estrogen excreted.

5. "Progesterone (or active corpus luteum secretion) facilitates the conversion of estrone to estriol."

Since mainly colorimetric determinations were used in this work and we have come to realize the difficulty of accurate analysis of estrogens in extracts of urine by colorimetric procedures, a reinvestigation of this problem is desirable.

In our experiments in which quantities of the order of 1 mg. of estrone or β-estradiol were administered to rabbits, the urines had practically no estrogenic activity. When estriol was given about 2 per cent was recovered in the urine.

In some other experiments, in which in contrast to those just mentioned, massive quantities of estrogens were administered to rabbits, Stroud (72) isolated estrone and another estrogen which was not fully identified but was probably β-estradiol following the administration of estrone. Subsequently, Fish and Dorfman (23) as well as Heard, Bauld and Hoffman (35) clearly showed that ovaries and uterus are not necessary for the conversion of α-estradiol to estrone and β-estradiol. The amount of estrogen found in the urine in these experiments was only a small proportion of that administered.

In another species, the guinea pig, Fish and Dorfman (22) found that both males and ovariectomized females convert α-estradiol dipropionate to estrone, thereby showing that this reaction can occur without the intervention of the ovary. The estrogen of the non-ketonic fraction was not characterized and consequently its weight is unknown but the estrone was equivalent to a recovery of only 4 per cent of the amount administered.

Experiments by Zondek (82), Dingemanse and Laqueur (13) and Dorfman (19) have shown that the rat has the capacity to effect almost complete destruction of estrogens.

Catabolism of estrogens by monkeys. The monkey, too, eliminates in the urine only a small proportion of administered estrogen, Frank et al. (26) finding about 20 per cent and Westerfeld and Doisy (78) less than 5 per cent. In the work of the latter investigators both estrone and α-estradiol were administered to a normal, an ovariectomized and an ovariectomized-hysterectomized animal. With either compound both ketonic and non-ketonic estrogens were excreted and there was little difference in the amounts excreted by the different animals.

To test the possibility that estriol might be more resistant to catabolism than estrone or α-

Metabolism of estriol

	OVARIECTOMIZED-HYSTERECTOMIZED MONKEY			UNOPERATED FEMALE		
Days of expt.	Estriol	Estrone	Estradiol**	Estriol	Estrone	Estradiol**
	γ	γ	γ	γ	γ	γ
1-5*	633	4	1.4	505	4	0.72
5-20	26,500	2	45	7,813	4	76
20-24	6,825	1.5	2	10,050	1.5	6
24-59	500	3	15	12,500***	6.9	2

* 100 mg. estriol injected daily.

** Values calculated as α-estradiol.

*** Urine collection stopped on 39th day.

estradiol each of two monkeys was given daily 100 mg. of powdered estriol in aqueous suspension by injection for a period of five days. The conjugated estrogens were hydrolyzed by boiling with HCl and the phenolic estrogens fractionated into the estriol, estrone and estradiol components by the scheme (16) previously reported. Only minute amounts of estrogenic activity were found in the estrone and estradiol fractions and there is no good evidence that these fractions owed their potencies to those compounds. However, purification of the estriol fraction yielded pure crystalline estriol equivalent to a large proportion of the estrogenic activity of that fraction. Though less than 7 per cent of the estriol was excreted in the urine, an appreciable quantity was still being excreted 20 days after the termination of the injections.

Owing to the interest recently created in β-estradiol by Stroud (72), Fish and Dorfman (23) and Heard et al. (35) the ovariectomized-hysterectomized monkey was given by injection during a three day period an aqueous suspension of 277 mg. of pure β-estradiol. The conjugated estrogens were hydrolyzed by boiling with HCl and the total estrogens fractionated according to our usual procedure. According to the partition ratios α- and β-estradiol should appear almost quantitatively in the same fraction. In this experiment unmistakable estrogenic activity was found in both the estriol and estrone fraction. However, the loss of estrogenic compounds amounted to about 99 per cent of the β-estradiol thereby indicating clearly that β-estradiol is not an important end-product of estrogen catabolism.

Metabolism of β-estradiol

Ovariectomized-hysterectomized monkey. 277 mg. of β-estradiol given by subcutaneous injection of an aqueous suspension.

DAYS OF EXPT.	ESTRIOL	ESTRONE	ESTRADIOL*
	γ	γ	γ
1–30	126	500	125
30–60	30	6	63

* Calculated as α-estradiol.

If the estrogenic potencies of column 4 had been due to β-estradiol the values by our method of assay would have been twenty times greater.

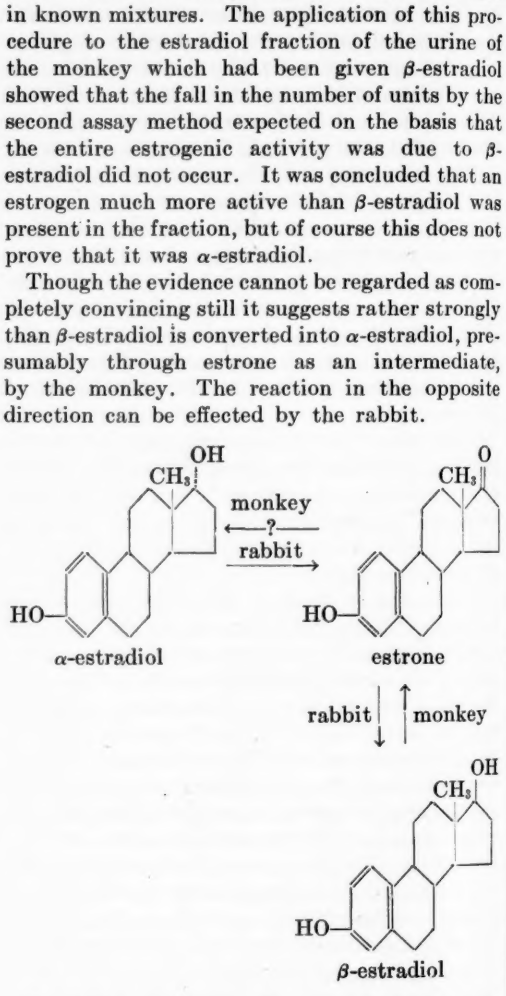
Since the existence of α-estradiol among the products of excretion would indicate the transformations

β-estradiol → estrone → α-estradiol

by the monkey, a study of possible reactions for the detection of minute quantities of α-estradiol was undertaken.

Assay of mixtures of α- and β-estradiol showed that the estrogenic activities are additive. Then by a differential mouse assay procedure in which the total estrogenic potency is determined by two methods—three injections of an aqueous solution and the other, one injection of a solution in oil—the amounts of α- and β-estradiol were determined in known mixtures. The application of this procedure to the estradiol fraction of the urine of the monkey which had been given β-estradiol showed that the fall in the number of units by the second assay method expected on the basis that the entire estrogenic activity was due to β-estradiol did not occur. It was concluded that an estrogen much more active than β-estradiol was present in the fraction, but of course this does not prove that it was α-estradiol.

Though the evidence cannot be regarded as completely convincing still it suggests rather strongly than β-estradiol is converted into α-estradiol, presumably through estrone as an intermediate, by the monkey. The reaction in the opposite direction can be effected by the rabbit.



Catabolism of estrogens by man. Following the administration of estrogens to women only a small percentage was found in the urine (26, 45, 55, 63, 68). Even after taking into account the excretion in the feces (Dohrn and Faure, 15; Siebke, 65, and others) approximately 80 per cent of the estrogenic activity had disappeared.

In an experiment performed by Heard and Hoffman (37), 250 mg. of α-estradiol were given to a man during an eight day period. Sixteen and two-tenths milligrams of estrone and 9.8 mg. of α-estradiol, a total of only 10 per cent of the administered estrogen were isolated from the urine. The urine was examined for estranediol but this compound was not detected. Since estriol occurs in human pregnancy urine and since

Fish and Dorfman (23) and Heard and his associates (35) have shown that the rabbit converts α-estradiol to the β form, it seems possible that some of these compounds although not in appreciable amounts might have been present in the urine.

The administration of estrogens to all of the common laboratory animals, to the gelding (Deulofeu, 12) and to man leads mainly to such profound changes in the estrogen molecule that the products of catabolism have negligible or no estrogenic action. Consequently, if further information is to be obtained it will be desirable to postulate changes in the molecule which seem possible on biochemical grounds and then examine the urine and feces for the hypothetical products.

Site of catabolism of estrogens. Although some of the earlier papers (66) report that blood inactivates estrogens, later work (Israel, Meranze and Johnston, 43) has cast doubt on the accuracy of those observations. In his study of brei from rat tissues, Zondek (82) found that liver produces rapid destruction, the spleen and kidney slowly produce inactivation but muscle, lung, brain, adrenals and testis have no effect. Recently, Heller (38) has repeated and extended these experiments. α-Estradiol and estrone were completely destroyed by liver but estriol was not appreciably affected.

By perfusion of a heart-lung preparation of a dog with blood containing estrogen, Israel, Meranze and Johnston (43) found little or no loss of estrogenic activity. If the preparation was altered so that blood was also passed through the liver rapid loss of estrogen took place.

In living animals additional evidence has been obtained which indicates that the liver plays an important rôle in catabolism of estrogens (Biskind, 5; Golden and Sevringhaus, 30; Talbot, 73; and Pincus and Martin, 59). Possibly the effect is due both to extensive alteration of the molecule and to the production of conjugated products having much less estrogenic activity.

In vitro oxidation of estrogens. The destruction of estrogens by liver brei, first observed by Zondek (82) and ascribed to an "estrinase" was confirmed by Heller (38) who in addition found that NaCN prevented the destruction presumably through its inhibiting effect on oxygenases. Recently, Graubard and Pincus (31) reported that extracts of rabbit liver and cytochrome failed to cause an appreciable uptake of oxygen by water-soluble estrogen esters.

Westerfeld (76) found that an extract of mushrooms produced inactivation of estrogen and Graubard and Pincus (31) showed that laccase from mushrooms causes an uptake of about 1 atom of oxygen per mole and a loss of 90 per cent of the estrogenic potency. Potato tyrosinase caused the uptake of 3 to 4 atoms of oxygen and complete loss of activity.

Since oxidations by hydrogen peroxide have frequently been assumed to resemble in vivo oxidative processes, Westerfeld (77) studied the effect of hydrogen peroxide on estrone. Depending on the procedure used, oxidation of either the A or D ring was obtained. Oxidation of the D ring gave an hydroxy monocarboxylic acid, the lactone of which was isolated and characterized.

The in vitro experiments with enzymes and with hydrogen peroxide together with the failure to account for more than 25–30 per cent of the administered estrogen warrant the working hypothesis that the estrogen molecule probably undergoes extensive change during its metabolism. Perhaps, an examination of the ether extract of urine in which a search of the neutral fraction for oxidation products of phenols and of the acidic fraction for acids related to the estrogens might be productive.

REFERENCES

(1) Allan, H. and E. C. Dodds. Biochem. J. **29:** 285, 1935. (2) Allen, E. and E. A. Doisy. J. A. M. A. **81:** 819, 1923. (3) Bachman, C. Am. J. Obst. and Gynec. **42:** 599, 1941. (4) Beall, D. Nature **144:** 76, 1939. (5) Biskind, G. R. Endocrinology **28:** 894, 1941. Proc. Soc. Exper. Biol. and Med. **47:** 266, 1941. (6) Bloch, K. and D. Rittenberg. J. Biol. Chem. **143:** 299, 1942. (7) Browne, J. S. L. Cited from J. B. Collip, Proc. Calif. Acad. Med. **1:** 38, 1931. (8) Butenandt, A. Naturwissenschaften **17:** 878, 1929. (9) Cohen, S. L. and G. F. Marrian. Biochem. J. **30:** 57, 1936. (10) Cohen, S. L., G. F. Marrian and M. C. Watson. Lancet **1:** 674, 1935. (11) Corner, G. W. Bull. Johns Hopkins Hosp. **67:** 407, 1940. (12) Deulofeu, V. Compt. rend. Soc. biol. **130:** 458, 1939. (13) Dingemanse, E. and E. Laqueur. Am. J. Obst. and Gynec. **33:** 1000, 1937. (14) Dingemanse, E. and R. Tyslowitz. Endocrinology **28:** 450, 1941. (15) Dohrn, M. and W. Faure. Klin. Wchnschr. **7:** 943, 1928. (16) Doisy, E. A. Female sex hormones. Univ. of Penn., Bicentennial Conference, 1, 1941. (17) Doisy, E. A., S. A. Thayer, L. Levin and J. M. Curtis. Proc. Soc. Exper. Biol. and Med. **28:** 88, 1930. (18) Doisy, E. A., C. D. Veler and S. A. Thayer. Am. J. Physiol. **90:** 329, 1929. (19) Dorfman, R. I. J. Biol. Chem. **119:** xxv, 1937. (20) Dorfman, R. I. Science **92:** 585, 1940. (21) Fieser, L. F. Chemistry of natural products related to phenanthrene, 2nd ed., 1937. Reinhold Publishing Corporation, New York. (22) Fish, W. R. and R. I. Dorfman. J. Biol. Chem. **140:** 83, 1941. (23) Fish, W. R. and R. I.

DORFMAN. J. Biol. Chem. **140:** xl, 1941; **143:** 15, 1942. (24) FRANK, R. T. J. A. M. A. **97:** 1852, 1931. (25) FRANK, R. T. Proc. Soc. Exper. Biol. and Med. **31:** 1204, 1934. (26) FRANK, R. T., M. A. GOLDBERGER AND F. SPIELMAN. Proc. Soc. Exper. Biol. and Med. **29:** 1229, 1931. (27) GALLAGHER, T. F., D. H. PETERSON, R. I. DORFMAN, A. T. KENYON AND F. C. KOCH. J. Clin. Investigation **16:** 695, 1937. (28) GIRARD, A. Bull. Soc. Chem. Biol. **15:** 562, 1933. (29) GIRARD, A., G. SANDULESCO, A. FRIDENSON AND J. J. RUTGERS. Compt. rend. Acad. Sci. **194:** 909, 1932; A. GIRARD, G. SANDULESCO, A. FRIDENSON, C. GAUDEFROY AND J. J. RUTGERS. Ibid. **194:** 1020, 1932; A. GIRARD, G. SANDULESCO, A. FRIDENSON AND J. J. RUTGERS. Ibid. **195:** 981, 1932. (30) GOLDEN, J. B. AND E. L. SEVRINGHAUS. Proc. Soc. Exper. Biol. and Med. **39:** 361, 1938. (31) GRAUBARD, M. AND G. PINCUS. Proc. Natl. Acad. Sci. **27:** 149, 1941; Endocrinology **30:** 265, 1942. (32) GULDBERG, E. Acta Obstet. Gyn. Scand. **15:** 343, 1936. Cited from G. W. Corner, Physiol. Rev. **18:** 169, 1938. (33) GUSTAVSON, R. G. Chapter XIV. Sex and internal secretions. E. Allen, C. H. Danforth and E. A. Doisy. The Williams & Wilkins Company, Baltimore, Md. (34) HART, G. H. AND H. H. COLE. Am. J. Physiol. **109:** 320, 1934. (35) HEARD, R. D. H., W. S. BAULD AND M. M. HOFFMAN. J. Biol. Chem. **141:** 709, 1941. (36) HEARD, R. D. H. AND M. M. HOFFMAN. J. Biol. Chem. **135:** 801, 1940. (37) HEARD, R. D. H. AND M. M. HOFFMAN. J. Biol. Chem. **141:** 329, 1941. (38) HELLER, C. Endocrinology **26:** 619, 1940. (39) HIRSCHMANN, H. AND O. WINTERSTEINER. J. Biol. Chem. **122:** 303, 1937–38. (40) HOLL, G. Ztschr. f. Chir. **226:** 277, 1930. (41) HUFFMAN, M. N., D. W. MACCORQUODALE, S. A. THAYER, E. A. DOISY, G. W. SMITH AND O. W. SMITH. J. Biol. Chem. **134:** 591, 1940. (42) HUFFMAN, M. N., S. A. THAYER AND E. A. DOISY. J. Biol. Chem. **133:** 567, 1940. (43) ISRAEL, S. L., D. R. MERANZE AND C. G. JOHNSTON. Am. J. Med. Sci. **194:** 385, 1937. (44) DE JONGH, S. E., S. KOBER AND E. LAQUEUR. Biochem. Ztschr. **240:** 247, 1931. (45) KEMP, T. AND K. PEDERSON-BJERGAARD. Endokrinologie **13:** 156, 1933. (46) KEMP, T. AND K. PEDERSON-BJERGAARD. Lancet **2:** 842, 1937. (47) KOCH, F. C. Chapter XII. Sex and internal secretions. E. Allen, C. H. Danforth and E. A. Doisy. The Williams & Wilkins Company, Baltimore, Md. (48) KUNDE, M. M., F. E. D'AMOUR, A. J. CARLSON AND R. G. GUSTAVSON. Am. J. Physiol. **95:** 630, 1930. (49) LONGWELL, B. B. AND F. S. MCKEE. J. Biol. Chem. **142:** 757, 1942. (50) MARKER, R. E. J. Am. Chem. Soc. **60:** 1725, 1938. (51) MARKER, R. E., E. ROHRMANN, E. J. LAWSON AND E. L. WITTLE. J. Am. Chem. Soc. **60:** 1901, 1938. (52) MARRIAN, G. F. J. Soc. Chem. Ind., London **49:** 515, 1930. (53) MARRIAN, G. F. Ergeb. Vitamin- und Hormonforschung **1:** 419, 1938. (54) MATHER, A. Proc. Am. Soc. Biol. Chem., J. Biol. Chem. **133:** lxiii, 1940. (55) MAZER, C. AND S. L. ISRAEL. J. A. M. A. **108:** 163, 1937. (56) MOON, H. D. Proc. Soc. Exper. Biol. and Med. **37:** 36, 1937. (57) NELSON, W. O. Anat. Rec., Suppl. **81:** 97, 1941. (58) PINCUS, G. Cold Spring Harbor Symposia on Quantitative Biology **5:** 44, 1937. (59) PINCUS, G. AND D. W. MARTIN. Endocrinology **27:** 838, 1940. (60) PINCUS, G. AND R. A. ZAHL. J. Gen. Physiol. **20:** 879, 1937. (61) VON PROBSTNER, A. Endokrinologie **8:** 161, 1931. (62) REICHSTEIN, I. T. Helv. chim. Acta **19:** 223, 1936. (63) ROBSON, J. M., T. N. MACGREGOR, R. E. ILLINGWORTH AND N. STEERE. Brit. M. J. **1:** 889, 1934. (64) SCHACHTER, B. AND G. F. MARRIAN. J. Biol. Chem. **126:** 663, 1938. (65) SIEBKE, H. Zentralbl. f. Gynak. **54:** 1734, 1930. (66) SILBERSTEIN, F., K. MOLNAR AND P. ENGEL. Klin. Wchnschr. **12:** 1694, 1933. (67) SIMPSON, S. L. AND C. A. JOLL. Endocrinology **22:** 595, 1938. (68) SMITH, G. V. S. AND O. W. SMITH. Am. J. Physiol. **98:** 578, 1931. (69) SMITH, G. V. S. AND O. W. SMITH. Am. J. Obst. and Gynec. **39:** 405, 1940. J. Clin. Endocrinology **1:** 470, 1941. (70) SMITH, G. V. S., O. W. SMITH AND G. PINCUS. Am. J. Physiol. **121:** 98, 1938. (71) SMITH, O. W. AND G. V. S. SMITH. Endocrinology **28:** 740, 1941. (72) STROUD, S. W. J. Endocrinology **1:** 201, 1939. (73) TALBOT, N. B. Endocrinology **25:** 601, 1939. (74) WADE, N. J. AND L. A. HASLEWOOD. Endocrinology **28:** 624, 1941. (75) WERNER, A. A. Int. Abst. Surg. **73:** 49, 1941. (76) WESTERFELD, W. W. Biochem. J. **34:** 51, 1940. (77) WESTERFELD, W. W. J. Biol. Chem. **140:** cxxxviii, 1941. (78) WESTERFELD, W. W. AND E. A. DOISY. Ann. Int. Med. **11:** 267, 1937. (79) WESTERFELD, W. W., D. W. MACCORQUODALE, S. A. THAYER AND E. A. DOISY. J. Biol. Chem. **126:** 195, 1938. (80) WINTERSTEINER, O., E. SCHWENK AND B. WHITMAN. Proc. Soc. Exper. Biol. and Med. **32:** 1087, 1935. (81) WOOLLEY, G., E. FEKETE AND C. C. LITTLE. Proc. Natl. Acad. Sci. **25:** 277, 1939. (82) ZONDEK, B. Skand. Arch. f. Physiol. **70:** 133, 1934; Lancet **2:** 356, 1934.

NUTRITIONAL PROBLEMS IN RELATION TO THE NATION'S HEALTH

JOHN R. MURLIN

University of Rochester School of Medicine and Dentistry, Rochester, N. Y.

Ten years from now the nutritional historian of the period through which we are passing may be imagined to write as follows:

"The year 1941 marked the beginning of two great wars, each started by a sneak attack. Both attacks were long in preparation, both should have been detected sooner. The one assailed the political and territorial integrity of the nation—the other its health. The Jap with perfidy and stealth concealed a dagger beneath a friendly gesture; malnutrition lurked in every loaf of bread."

"The National Nutrition Conference called by the President in May of that year was the occasion by which the true state of affairs regarding nutrition was brought into the open. This conference was made necessary because a previous one called ten years earlier and known as the White House Conference on Child Health and Protection did not wholly finish its job. That Conference resulted in the publication of some 20 volumes of proceedings in which the word malnutrition occurred very few times and its total discussion occupied less than 10 pages out of some 8000. However, in one of the volumes devoted to education and training is found a notation that along with an estimated 1,000,000 children between the ages of 5 and 18 years who had defects of speech, another 1,000,000 who had damaged hearts, 3,000,000 who had impaired hearing, there were some 6,000,000 who were described as 'malnourished.' The facts didn't register at all."

"After many meetings of sections and two large meetings in Washington, the White House Conference filed its valuable reports and dissolved feeling it had accomplished great things. At least it had brought together the knowledge of the time adequately—even handsomely. The volume on Nutrition summarized by far the largest part of the nutrition of children as it is practised today by the most intelligent pediatricians. It records proof of an anti-pellagra vitamin but calls it vitamin G. It discusses quite at length the rôle of iodine and iron in nutrition and devotes a chapter to 'physiologically rare elements,' listing as such, aluminum, cobalt, nickel, copper, fluorine, manganese, silicon, and zinc. Other volumes were equally impressive and complete, and the delegates had reason to feel proud of their accomplishments."

"What though the Conference occurred in the pit of the depression, the expenses of the delegates were paid out of the National treasury and all was well with the world, for prosperity was 'just around the corner.'" Here I leave the rôle of historian and speak for myself.

The conference of May 1941 had quite a different tone. It was brought together to meet a challenge and although many felt it was all too beautifully streamlined and engineered to come out at the right exit, it did not adjourn with a smile of satisfaction so much as with a frown of determination. The major premises of fact none questioned seriously; for they were taken from the very impressive studies of Dr. Hazel Stiebeling and her several associates. Because I have found that relatively few members of this Federation seem to be acquainted with these studies, I am giving the following few statements taken verbatim from the article on Nutrition in the 1941 volume of Annual Reviews of Biochemistry by Doctors Stiebeling and Leverton. They say:

"In 1940 there came from the press the first of the volumes that present the detailed information on food consumption that was obtained in a nation-wide coöperative study undertaken in 1936 by five Federal Agencies—the Department of Agriculture, the Department of Labor, the National Resources Planning Board, the Central Statistics Board and the Works Progress Administration. . . . The facts were obtained from non-relief native-born families in Chicago, New York, 6 other large cities, 14 middle-sized cities, 29 small cities, 140 villages and 66 farm counties located in various parts of the country."

In general it was found that, "diets of low money value ($1.38 to $2.07 per food expenditure unit per week) were found to be of poor nutritive quality in every community studied. . . ."

"At a higher level of money value ($2.08 to $2.76 per week per food expenditure unit) the average nutritive values of diets of groups of families were such as to suggest a fairly satisfactory although not generous food supply. . ."

"As village and city families had more money to spend for food, the foods they bought provided increased quantities of every nutrient considered, although the rate of increase was not the same for all nutrients. It tended to be least for calories and greatest for ascorbic acid. This shows that per capita consumption of inexpensive sources of calories, such as grain products, potatoes, sugars, and fats other than butter, increased but little as food expenditures rose, whereas the per capita consumption of fresh fruits and vegetables increased markedly."

"With the income distribution and food price levels similar to those prevailing in 1935-36 it

seems probable that at any one period about one-fourth of the families of the United States would be found to have good diets according to the specifications listed, more than a third fair diets and another third or more poor diets."

The authors, of course, state the amounts of several of the key nutrients contained in what they call poor, fair, and good diets.

These results in the United States were strikingly confirmed by the findings of McHenry (1) published the same year as the volume referred to, namely, 1940, on diets in Canada. Urban families having incomes of less than $1000 a year obtained only 76.5 per cent of the calories contained in the Canadian Standard, 77 per cent of the protein, 69 per cent of the calcium and 62 per cent of the iron. Urban families having incomes from $1500 to $2400 per year got 93 per cent of the calories in the Canadian Standard, 98 per cent of the protein, 116 per cent of the calcium and 98 per cent of the iron. He concludes that "the Canadian Standard therefore is easily attained."

If we define malnutrition as the result of a diet qualitatively so unsatisfactory as to produce measurable deficiencies, and reserve the word undernutrition for the diet which is lacking only in quantity, evidence of its existence has been found in recent years in South Africa (2, 3), Bermuda (4), New Zealand (5), India (6), the Colonial Empire (7), Ireland (8), England (9), Germany (10), Japan (11) and Italy (12). It is noteworthy, however, that while the democratic countries have steadily revealed the bad state of their nutrition, the most recent reports from Germany, Japan and Italy seem boastfully optimistic. Certainly this is true of Germany.

The surveys conducted in this country seem to be much more extensive and account has been taken of more of the essential nutrients; hence, if greater emphasis is placed here on malnutrition, it is partly due to a better appreciation of the importance of many nutrients (particularly the several members of the B complex) and partly to the freedom with which our investigators may report their findings without any suggestions from government.

Note in passing this report from the Philippines (13). Investigators working under the auspices of the National Research Council found that as a result of improper nutrition, the physical standard of the greater part of the population is "appallingly low." The authors review measures taken to alleviate nutritional distress in other parts of the world with particular reference to labor camps in South Africa and to the work of the Imperial Government Institute for Nutrition in Tokyo which three times a day *provides for factory workers a scientifically prepared meal, packed in cans and delivered at the factories at a very low cost.* Contrast with this a report from Italy, that *Il Duce* had conferred with the director of rabbit culture looking to the production of more rabbit meat.

The National Nutrition Conference was composed of 9 sections all of which drew up recommendations for action. This word *"action"* was emphasized many times as the keynote. A bad situation exists which may become worse; something must be done about it. The delegates were requested to delineate the objectives and the government would do the rest. The present time is too early to evaluate the results of this conference, and the present speaker is not the person to do so. At least we can say that no great row of books has been published, but a great deal of popular interest has been aroused. Refresher courses in nutrition are being given in many Universities (we have one just now with 244 registrants) and a considerable amount of research has been stimulated.

Section I of the conference was given over to the subject of research under the leadership of Professor McCollum. It listened to many suggestions, pondered over their relative importance *for defense*, and through its chairman offered eight fields of inquiry as follows:

1. Improvement of presently known chemical and biological procedures for estimating the amounts of the essential nutrients in foods and their physiological availability.
2. More refined techniques for the detection of nutritional deficiency states.
3. More precise determination of the optimum and minimum requirements of human subjects for each of the nutrients.
4. Study of problems relating to the nutritional needs of the individual as influenced by constitutional inefficiencies, by suboptimal nutrition, by disease and convalescence.
5. Studies directed toward clear definition of the physical status of the individual.
6. Study of all factors affecting the nutritive value of foods and their preservation during the interval between production and consumption.
7. Study of methods of preparation of foods for consumption so as to avoid losses of nutrients.
8. Food habits and methods and effects of changing them.

I call these to the attention of this gathering in the hope that some of you may find yourselves in position to take up or continue your investigations in some of these general subjects.

The section on Research also offered a resolution calling for the appointment of a Committee by the Conference which should survey existing facilities and resources both material and personal in land-grant colleges and universities qualified to engage in or continue research efforts along the lines indicated in the recommendations. This was not done by the Conference, but a subcom-

mittee on research was nominated by Dr. Russell M. Wilder, Chairman of the Committee on Food and Nutrition, to the officers of the National Research Council with duties somewhat akin to the committee named in the resolution. This subcommittee, later denominated a full committee, after reconstitution of the Committee on Food and Nutrition as a Board, soon began reconnoitering the different sectors of the nutritional front. Its duty was to sort out problems which seemed most important for defense and to invite through the chairman of the Food and Nutrition Board those apparently best qualified to take up or continue lines of work which might contribute most to the principal objectives of the main problems. The participation of 46 individuals or groups had been thus invited up to February 14, 1942. Twenty-one individuals or groups at once consented and went to work. Others stated they could not do so without financial aid. In the latter cases, the individual or group was invited to present a definite project with the necessary budget for personnel and materials, and The Committee would review each project submitted endeavoring to evaluate it with reference to its importance in view of the emergency and report to the officers of the National Research Council.

That was before Pearl Harbor. Since then the attitude of government agencies supplied with funds for research very naturally has been to give decided preference to, or even consider only, those projects most definitely and directly related to the war effort. The foremost consideration everybody feels, must be given to the nutrition of the military forces, and suggestions of the most pressing problems in this area are now coming from those best qualified to supply them. Projects relating to the nutritional needs of industrial workers or others directly contributing to the war effort doubtless properly come next in order.

The Committee on Research of the Food and Nutrition Board is now reëvaluating some of the earlier projects received. The duty of the Committee, however, is purely advisory as are all committees of the National Research Council. Its recommendations are transmitted to the Chairman of the Division of Biology and Agriculture of which the Food and Nutrition Board is a part and through the chairman they may be sent for implementation to the Division of Nutrition of the Office of Defense, Health and Welfare Services of the Federal Security Agency, or to the Committee on Medical Research of the Office of Scientific Research and Development. All of this sounds very complicated, but close liason exists between the bodies named and little time is lost. It seems in order to make known to this body of scientists so many of whom are experienced in nutritional research, the names at least of the offices and agencies concerned with the research side of nutrition. On the practical side of application to the Nation's needs, mention should be made of the Farm Security Agency, the Federal Food Stamp Plan of the Surplus Marketing Administration and the Bureau of Home Economics—all under the United States Department of Agriculture. Attendance at the meetings of the Food and Nutrition Board has been especially rewarding because of the glimpses one gets of the splendid work of these groups in the Department of Agriculture in meeting nutritional needs where they are most serious. Whatever one may think of the economic policies of the New Deal, one learns to respect the great body of scientific and humanitarian workers devoting themselves to the nutritional welfare of us all.

But what of the relation of these nutritional matters to the nation as a whole? Nobody doubts that nutrition is related to health, but we of this Federation have become so conditioned to the experimental method that we would like to see it demonstrated first on the rat.

Very well, consider these experiments on rats at the University of Toronto by Robertson and Tisdall (14). Rats were fed a measured amount of *Salmonella muriotitis* called the rat typhoid organism. The effect of several nutrients added to diets deficient in each successively, and the percentage of survivors after 28 days on control and supplemented diets respectively were as follows: I read first the percentage of survivors on the diet with adequate nutrient, and second the percentage on the deficient diet.

Percentage of survivors

NUTRIENT	SUPPLEMENTED DIET	DEFICIENT DIET
Vitamin A	79	40
Vitamin B Complex	72	20
Vitamin D	55	28
Minerals	87	54
Protein:		
Casein	90	
Wheat gluten		57

Granting that all factors necessary to equalize conditions except for the one variable were included, every nutritionist would accept these results as good evidence for the rat that each of the nutrients studied does positively affect resistance to an infectious disease. Most students of the problem whose works are reviewed by Clausen (15) in the A.M.A. book on "The Vitamins" agree, I think, that while vitamin A does not prevent acquiring infectious diseases, it does alter the resistance, course of the disease, and survival. A new review by Tissue (16) reports that a study of

the diet of 100 cases of the childood type of tuberculosis, showed that many of the children had been on grossly deficient diets. Twenty-four per cent of the dietaries analyzed were deficient in first class protein, 63 per cent deficient in milk and other sources of vitamin A, while 85 per cent were deficient in fruit and vegetables as sources of vitamin C. Faber (17) has shown that infant mortality from tuberculosis in Denmark moved parallel with margarine consumption and inversely with milk consumption. From vital statistics of the disease in Greenland also Faber concludes that the association of relative immunity appears to be with a diet high in foods of animal origin, for on their natural diet the Eskimos exhibit a benign type of the disease, but where imported food (chiefly carbohydrate) forms a substantial part of the diet, it becomes acute.

It is encouraging to learn that more and more surgeons are becoming aware of the importance of preoperative attention to nutrition. A striking illustration comes from our host city. Stewart (18) gives specific details of a routine management in obstructive jaundice which resulted in a reduction of the mortality rate to one-sixth its previous level at the Massachusetts General Hospital. A high carbohydrate, low-fat diet with adequate supplements of all vitamins including K was maintained for 5 to 8 days before operation. Drummond (19) suggests that the modern susceptibility to appendicitis may be due to a significant extent to deficiency of the B complex and particularly B_1.

Thus in improving resistance to infection and the conservation of lives from surgical risk nutrition with its complete panoply of nutrients is advancing into the hospitals where it is somewhat overdue in most services except pediatrics. Kollmann (20) reported in 1939 from Nürnberg that intramuscular injection of vitamin B_1 caused a rapid reduction of nutritional edema. Per oral administration doubtless would have accomplished the same result. Which leads one to protest against enormous doses of vitamins injected with a syringe instead of feeding the natural way, just to make it seem more professional, shall I say? What justification, for example, can be offered for doses up to 600,000 I.U. of vitamin D, as irradiated ergosterol, in the treatment of rickets (Vollmer, 21)?

We must not forget, however, that the great majority of people never go to the hospital. What are the nutritional problems the solution of which would contribute most at this critical time to the improvement and maintenance of health in the general population? I believe I correctly interpret the opinion of the Food and Nutrition Board in stating that the first problem at this time concerns preservation and conservation of nutrients in our natural foods, and the second relates to the determination of optimal intake of each nutrient substance for different physiological conditions.

The first of these so far as it concerns conservation in home processing has been taken up energetically by the experiment stations and the land-grant colleges under the leadership of Miss Sybil Smith, Principal Administrator of the Experiment Stations. They are ideally equipped and oriented to the communities in which they are located and to the local food crops to handle such investigations. The industrial processing of foods also is receiving more and more attention. Several milling companies both in Canada and the States are working actively on higher extraction flours. The Council on Foods and Nutrition of the American Medical Association last summer (22) listed three flours then available which showed assays of 2 or more mgm. of thiamine per pound. And one of our largest baking companies[1] has just announced that their principal white bread now contains more than the requirement of the Food and Drug Administration for enriched bread, obtained in part by higher extraction and in part by the use of high vitamin yeast.

This leads me to make just one comment about the enrichment program. So far as one can see it is a timely and proper emergency measure. But we must not be misled into thinking that bread enriched only with thiamin, niacin, and iron is the equivalent of whole wheat bread, or that adding riboflavin and calcium now optional ingredients would make it so. Doctor Nasset and his students in my department have shown that a dog is still deficient, exhibiting both skin and mouth lesions, on a basal diet free of B vitamins to which have been added thiamin, riboflavin, niacin, pyridoxin, pantothenic acid, choline and inositol. They are free of such lesions when fed on nothing but bread from wheat flour lacking only the epidermis of the grain, i.e., 98 per cent extraction. This experiment illustrates the fact that retention is better than enrichment. We do not believe, however, that the problem of getting people to eat whole wheat bread is an easy one. Other observations by Doctor Nasset and his students suggest that tolerance of whole wheat bread may be improved by the addition to it of more pantothenic acid and inositol. At all events, dogs fed on nothing but whole wheat bread evacuate the meal more rapidly from the stomach when either of these members of the complex is added and digest it more completely with the pantothenic acid supplement alone.

The observations of Ebbs, Tisdall and Scott (23) on the benefits to both the mother and her offspring from selection of more protective foods

[1] Continental Baking Co.

in the antenatal period, are very impressive and doubtless will be confirmed and extended. Other physiological conditions such as the strain and tension to which large sections of the population soon will be subjected call for similar studies. Indeed, I venture to suggest that the members of this Federation, because of the extra load of teaching which a large percentage of them will be obliged to undertake to carry through the acceleration program would do well to engage seriously in nutritional investigations in their own interest and that of their families. Improved knowledge of the materials which traverse the gullet three times a day might produce some astonishment and surely would do no harm. However, the speaker is not one of those who believe that meticulous attention to the diet alone can develop the toughness of fiber the times call for. Reasonable alternation of work and relaxation, diversion of the mind by vasomotor shift of cerebrally exhausted blood to muscles and kidney is just horse sense. In the old days of the stage coach the horse with most sense was chosen as the wheel horse. He anchored and guided the rest of the team. Wheel horses among men are in demand today.

REFERENCES

(1) McHenry, E. W. Canad. M. J. **31:** 584, 1940. (2) Le Riche, H. South African Counc. Educat. and Social Res. Ser. no. 13, 1940, pp. 158. Ref. in Nutr. Abs. Revs. **10:** 387, 1940. (3) Fox, F. W. South African J. Sci. **36:** 36, 1939. (4) Report on the State of Nutrition of School Children in Bermuda, 1939, 12 pp. H. M. S. O. (5) Wilson, E. C. G. Ann. Rept. Nutr. Com., Med. Res. Counc. New Zealand, 1939–40, 9–57. Ref. in Nutr. Abs. Revs. **11:** 293, 1941. (6) Mitra, K. Palna J. Med. **14:** 223, 1939. (7) Economic Advisory Council, Com. on Nutr. in the Colonial Empire, H. M. S. O., 1939, pp. 210 and 146. Ref. in Nutr. Abs. Revs. **9:** 692, 1940. (8) Deeny, J. Poverty as a cause of ill health. Statistical and Social Inquiry Soc. of Ireland, 1940, pp. 10. (9) Brockington, F. Pub. Health **52:** 307, 1939. (10) Bickel, A. Deutsch. Med. Wchnschr. **66:** 393, 1940. H. Hoesslin. Ernährung **5:** 88, 1940. W. Pfannenstiel. Öffentl. Gesundheitsd. **4:** 913, 1939. (11) Teruoka, A. Rep. Japan Inst. Sci. Lab. Inc. No. 43, 1939. Ref. Nutr. Abst. Revs. **9:** 1009, 1940. (12) Aiello, G. Problema alimenta **2:** 1, 1938. (13) San Augustin, G. and J. Albert. Philippine J. Animal Indust. **6:** 247, 1939. (14) Robertson, E. C. and F. F. Tisdall. Nutrition and resistance to disease, nutrition in every day practise. Publ. by Canad. M. A. J., 1939, p. 62. (15) Clausen, S. W. Pharmacology and therapeutics of vitamin A. The vitamins. A. M. A. Publ., 1939, p. 54. (16) Tissue, K. A. J. Am. Dietetic Assoc. **16:** 313, 1940. (17) Faber, K. Ugeskr. Laeger. **101:** 1148, 1939. Ref. in Nutr. Abst. Revs. **9:** 706, 1940. (18) Stewart, J. D. J. Am. Dietetic Assoc. **17:** 535, 1941. (19) Drummond, J. C. J. Roy. Inst. Public Health Hyg. **2:** 44, 1939. (20) Kollman, A. Arch. Kinderheilk. **117:** 64, 1939. (21) Vollmer, H. J. Pediat. **14:** 491, 1939. (22) Nutritionally improved or enriched flour and bread. J. A. M. A. **116:** 2849, June 28, 1941. (23) Ebbs, J. H., F. F. Tisdall and W. A. Scott. J. Nutrition **22:** 527, 1942.

NEWER VIEWPOINTS ON CLINICAL ANESTHESIA

RALPH M. WATERS

Department of Anesthesia, Medical School, University of Wisconsin

Viewpoints are as variable as the minds of the viewers. Your request for newer viewpoints on clinical anesthesia in the Spring of 1942 may receive as an answer a restatement of attitudes manifest two years after the original announcement of surgical anesthesia (1).

John Snow was without doubt the father of the scientific administration of drugs to abolish pain. After a year of exhaustive study, of arduous work in the laboratory and of experience in the clinical administration of ether and another year similarly spent with chloroform, Snow wrote—in December, 1848 (2):

"When I state that I have administered chloroform in almost every possible condition in which a patient could require an operation, it must not be considered that I have acted without discrimination, but rather, that going on gradually, and acting on previous experience, supposed objections have one by one vanished, and it has appeared that care in the mode of giving the vapour was the main guarantee, both of safety and success. This view of the subject is entertained by others as well as myself; for, it must be recollected, that I have never given chloroform or ether in an operation without the concurrence of other medical men."

By "other medical men" he referred to such

associates as Mr. Liston at University College Hospital and Sir Benjamin Brodie at Kings College Hospital in London. I quote these words, written 94 years ago, because we clinical anesthetists failed to reread and consider them for a great many years after Snow's death. Until recent times, the search for new drugs and new gadgets occupied our effort. We failed to appreciate that a thorough understanding of the effects of the drugs which we use, upon the essential physiologic processes, was the foundation of "safe and successful" administration. In speaking of "newer viewpoints on clinical anesthesia," then, I am proud to announce the newer viewpoint broadly, in a manner which may appear to be a paraphrasing of Snow's statement:

To relieve pain "safely and successfully" we must first understand the physiologic processes which maintain transport of food and oxygen to—and carbon dioxide and other waste products from —the cells of the body. Secondly, we must understand the many other effects of depressant drugs, which accompany the relief of pain and which embarrass the transport system. Finally, in the light of such understanding, we must develop practical methods for the parallel administration of drugs and the protection of physiologic function in a manner which will assure both safety and success.

TABLE 1

Possible variables from the normal tensions of oxygen and carbon dioxide as a result of the action of drugs when combined with the inhalation of artificial atmospheres and artificial aids to respiratory exchange

O_2 normal	CO_2 normal
O_2 normal	CO_2 excess
O_2 normal	CO_2 deficit
O_2 excess	CO_2 normal
O_2 excess	CO_2 excess
O_2 excess	CO_2 deficit
O_2 deficit	CO_2 normal
O_2 deficit	CO_2 excess
O_2 deficit	CO_2 deficit

For much assistance in arriving at our present understanding, we are indebted to the individual membership which constitutes the Federation of the American Societies for Experimental Biology and their confreres in other parts of the world. For further aid in broadening the foundation of anesthesiology, we will often look to you. Your officers have seen fit to honor me with this opportunity of expressing the gratitude which every clinical anesthetist feels toward the Biologic Sciences. Perhaps I may do this by citing a very few of the many uses to which your contributions have been put in the hospital. In doing so, I shall deem it a privilege if I may pause here and there to hint at lines of investigation which might attract your further interest and eventuate in the more complete solution of problems which puzzle the practical anesthetist.

The anesthetist finds it advantageous to look upon the physiologic mechanisms involved in carrying oxygen, carbon dioxide and narcotic substances to and from the cells of the central nervous system as a single system of transport. Obviously it includes the contribution of respiration, circulation and the control of these exerted by the autonomic nerves. To assign transport deficiency in a particular patient to the proper element is often difficult. The disease suffered by the patient may not only modify a certain function but it may also modify the action of a narcotic drug upon that function. It is a rather common view that our failure to attain "safety and success" is most often due to our inability to secure from drugs adequate depression of the sensory pathways without undue embarrassment of transport. It should perhaps be emphasized that regardless of which anesthetic drugs are employed and whether they are administered orally, rectally, intravenously or injected into regions around the nerve roots or trunks, the resulting physiologic changes ultimately cause a transport deficit. The prevention or elimination of this deficit is the duty of the clinical anesthetist.

In spite of the overlapping of the function of respiration, circulation and the autonomic mechanism, I shall try to mention each system separately. I should like to finish my discussion with a reference to cooperative effort between the scientific investigator in the laboratory and the serious minded clinician in the hospital.

Respiration. Your warnings of the dangers of hypoxia have been heeded. We have "leaned over backward" in avoiding them. As is frequent in such circumstances, we have retreated only to step into trouble of another kind (3, 4, 5). Pure oxygen being abundantly available, it came to be used as an atmosphere for all depressed patients. The result was doubtless better than even minor degrees of hypoxia.

The clinical anesthetist administered artificial atmospheres to his patients for many years before he began to appreciate that he might produce eight types of variation from the normal tensions of oxygen and carbon dioxide in the alveoli, blood and tissues (see table 1). The old concept of asphyxia, with always an increase in carbon dioxide and a decrease in oxygen, had to be discarded when we began to administer artificially mixed atmospheres to patients who had had their respiratory activity depressed by drugs and who were subjected to artificial changes in ventilation. We are coming to appreciate that a patient with an obstructed airway or depressed respiratory exchange can, in spite of ample oxygen, suffer

from an excess of carbon dioxide in his tissues. When filled with pure oxygen, inactive portions of the lung may more readily become atelectatic than when they are filled with air. For these and other reasons, we seem to be assuming a more rational attitude toward the use of oxygen. We are becoming convinced of the necessity of an unobstructed passage to and from the alveoli and adequate ventilation through that passage, regardless of how "pink" the patient may be. As a result we have acquired skill in the placement of artificial breathing tubes, into the trachea if necessary (6), and skill in the artificial supplementation of respiratory exchange. Certainly many anesthetists are convinced that these mechanical aids to respiration are safer and more reliable than is the use of stimulant drugs (7, 8).

Carbon dioxide absorption technic (9, 10, 11, 12, 13, 14, 15). For the past twenty years, the chemical absorption of carbon dioxide from anesthetic atmospheres has become increasingly popular. Indications are that in military practice, advantages of economy will tend still further to popularize the application of this principle, not only to anesthetic but also to therapeutic atmospheres and those inhaled by aviators and submarine workers. There are many problems of a physical, chemical and engineering nature which need attention if the chemical absorption of carbon dioxide is to be made safe and successful. At the same time, most intriguing questions for the physiologist and the biochemist demand answers—*a*. The atmosphere inhaled in this technic is saturated with moisture and is warm. Why is perspiration much less often seen than when the same atmosphere is used with "open" technic? *b*. The mask and connections constitute as great, often greater, "dead space" between patient and the soda lime than is present between patient and exhalation valve with "open" technics. Carbon dioxide tensions in the blood of patients are above normal. Why is breathing quieter than that seen when similar atmospheres are inhaled by "open" technic? *c*. The blood pressure during the use of absorption technic is usually normal or above normal. Why is it that occasionally, following a long period of the use of this technic and more often when a high tension of oxygen has been employed, the sudden removal of the mask is followed by an extreme drop in blood pressure? (fig. 1.) Usually this hypotension is accompanied by a slow pulse and the absence of a typical "shock picture." However it may assume a more alarming aspect. It is less often seen following "controlled respiration" than after administrations in which the patient's unaided respiratory efforts maintain exchange. Is it a reaction to the sudden removal of a humid atmosphere, to the change from warm air to that which is cooler, or is it evidence of the inability of the organism suddenly to readjust to normal the biochemistry of the blood and tissues which have become accustomed to an environment containing high tensions of both oxygen and carbon dioxide?

Controlled respiration (16, 17, 18, 19). We have already emphasized the present belief in the importance of free breathing and adequate respiratory exchange. The anesthetist deals with patients whose vital functions are modified, however slightly, by the action of drugs. If the ventilation of such patients is augmented, either by increasing the volume of each inspiration, through pressure on the rubber breathing bag, or by subjecting the patient to the action of a mechanical respirator, respiratory muscle activity ceases. More recently this circumstance has been utilized, apparently to the advantage of surgeon and patient. The technic of controlled respiration,

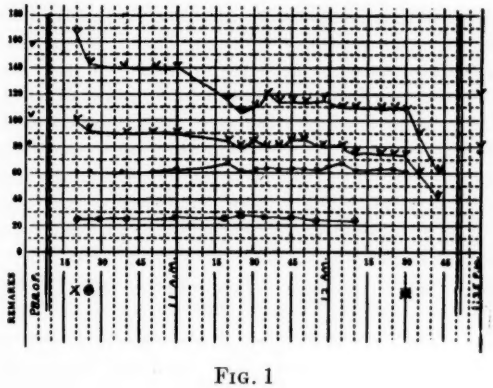


FIG. 1

conducted by simple pressure of the hand upon the breathing bag followed by quick release (thus providing ventilation barely adequate to prevent resumption of normal respiration) has been utilized at the Wisconsin General Hospital for the past several years in about ten per cent of all cases anesthetized by inhalation. The period of "control" has varied from a few minutes to a few hours as conditions demanded. The method has been used in many other places. The advantages are several. If the success of the anesthetic depends upon a dose of the agent which unduly depresses pulmonary ventilation, adequate exchange can be assured in this manner. If delicate surgical manipulations require a quiet operative field, movements of the lungs and diaphragm by manual exchange can be synchronized with periods when they are least embarrassing, or they may be temporarily omitted altogether. If the pleural cavity is open, ineffectual movements of the thoracic walls and viscera, resulting in both respiratory and circulatory embarrassment, can

be avoided. One may wonder also whether during certain operative procedures, it is not desirable to relieve a weak patient from the energy output necessary to contract the diaphragm and intercostal muscles.

Those unfamiliar with this technic tend to hyperventilate, thus risking trauma to the alveoli. However we once utilized an extreme exaggeration of this technic (20) to simulate conditions of hyperventilation previously studied in animals (21). The results were apparently interpreted by some readers (22) as being those of controlled respiration. They are mentioned here because of their bearing on trauma. By combining an extreme exaggeration of the inspiratory phase with a forced expiration produced by chest compression, we were able to reduce carbon dioxide tension of the blood to as low as 9 mm. Hg but no evidence of trauma or other ill effects could be detected in these patients during the following days. Since extreme effort was required in exaggerating the exchange in the experiments just mentioned, it seems unlikely that the method described as controlled respiration and used for clinical anesthesia, can result in significant pathologic or physiologic damage. Mechanical respirators, both of the inflation only, and two phase type, are being utilized to maintain "controlled respiration." Hence the physiologic and biologic changes and the possibility of structural injury deserve much further investigation. Clinical experience with the technic up to this time leads to the impression that it is valuable and justifiable, at least when performed by judicious manual manipulation of the breathing bag in the to-and-fro absorption technic.

Circulation. The various handicaps imposed upon the circulation by anesthesia tend at present to be attributed by the anesthetist to secondary results of the state of anesthesia rather than to direct action of drugs upon the organs of circulation. We have referred to the depression sometimes seen following the use of the "absorption" technic. Depression or obstruction of respiration modifies the normal rhythmic change of the negative pressure in the chest, a change which may be important in the movement of blood. Loss of muscle tone (skeletal and circulatory) has a similar effect. Loss of fluid together with hydrostatic influences is considered to be highly significant in the production of depressed states of the circulation which were formerly attributed to the direct action of the anesthetic drugs. Sudden excessive overdose of certain anesthetic agents is still considered as a possible cause of cardiac arrest although the more general feeling is that asystole occurs most frequently through the mediation of the autonomic system (23, 24). We are inclined to heed your warning that certain of the analeptics and sympathomimetic substances formerly thought to be useful in the treatment of circulatory inadequacy, may be highly dangerous in certain circumstances (25).

In spite of excellent contributions from the laboratory, the clinical anesthetist comes more and more to depend, for the management of circulatory depression, upon protection and aid of the transport mechanism, upon physical support, and upon replacement of food, fluid and biologic deficiencies rather than upon stimulant drugs. The judgement used during and following its administration rather than the choice of drug will, we believe, determine the absence of shock and shock-like states.

The autonomic system. The literature of the Biologic Sciences in recent years has been most generous in supplying us with information regarding the autonomic control of the vital functions. It may seem ungrateful to say that the result has been most confusing to the clinical anesthetist. We are free to admit that a part of the confusion is due to our inability completely to understand and correlate some of the published reports. Certainly you have convinced us that herein lies the explanation of many a clinical observation that has puzzled us in the past.

Perhaps our confusion may originate, in part, in certain peculiarities of the autonomic mechanism. The numerous elements in both the craniosacral and thoracolumbar functions are affected by illness and by drugs. The clinician sometimes wonders whether the drug effect may not be at variance from the laboratory findings when illness has preceded drug administration. In discussing respiration we have referred to the numerous variations which are possible in the tensions of oxygen and carbon dioxide. The clinical circumstances which we are encountering regarding the autonomic nervous system make us wonder if the variables here are not still further confounded. Variables of the respiratory gases were fairly obvious, once the possibilities were appreciated. Depressant drugs in general cause only one set of changes in gas tensions—a deficit of oxygen accompanied by an excess of carbon dioxide. Not so simple are the effects of depressant drugs upon the autonomic system.

On previous occasions I have referred to two patients whom we encountered some years ago. A description of them here is illustrative of the individual variability of response to drug action.

A woman, aged 38, was anesthetized to permit the removal of her appendix. She was considered rather "high strung" and unstable mentally. One thirty-second of a grain (0.002 gram) of dilaudid and one one hundred and fiftieth of a grain (0.000432 gram) of scopolamine hydrobromide were administered hypodermically one and

three quarter hours before induction of anesthesia with cyclopropane.

Pressure on the eyeballs was exerted during first plane, third stage anesthesia. Extreme slowing of the pulse rate resulted eventuating in asystole. Respiration was arrhythmic during stimulation but reverted to normal on release of the stimulus without artificial aid.

Electrocardiograms were then made of periods of complete cardiac arrest, dependent for duration upon the cardiologist's approval (fig. 2). The depth of anesthesia was varied between the first and third planes of the third stage, while tests of the oculo-cardiac and carotid reflex sensitivity were recorded. Hyper-response eventuating in asystole persisted. The mask was then removed until almost complete recovery from cyclopropane had occurred. Ether anesthesia was then induced and electrocardiograms made during the first three planes of third stage anesthesia. In each plane, similar hyperactivity of the two reflexes was evident. Ether anesthesia was then forced to the fourth stage with respiratory arrest. The hypersensitivity of the reflexes was abolished in this degree of ether depression.

After an interval of two weeks and when no drugs had been administered, the response to similar stimulation of the oculo-cardiac and carotid reflexes in this patient was found by the electrocardiologist to be normal.

During discussion of this case among the house staff, the following case came to light.

A man, aged 36, was reported to have had similar hypersensitivity of the oculo-cardiac and carotid reflexes to the extent of asystole while undergoing routine physical examination. The cardiologist verified the circumstance and recorded it. The tentative diagnosis had been "neurasthenia" and rheumatic heart disease with functional capacity of IIA was recorded. No indication for a surgical procedure was evident at that time. Two years later, however, a secondary repair of a right inguinal hernia was performed. Anesthesia was induced without any previous medication. Nitrous oxide was used for the first five minutes, cyclopropane for twenty-five minutes and then ether for the remainder of the operation. The sensitivity of the oculo-cardiac and carotid reflexes, as recorded in the electrocardiogram, was found to be normal during tests throughout a period prolonged to three hours. One twelfth of a grain (0.0054 gram) of morphine sulphate and one three hundredth of a grain (0.0004 gram) of scopolamine hydrobromide was added to the inhalation, intravenously, at the end of the second hour and one one-hundredth of a grain (0.00065 gram) of scopolamine hydrobromide just prior to wakening. No evidence of effect on the reflex mechanisms was noted as a result of these intravenous injections.

The contrast in these two cases is striking. One gave no evidence of extreme hyperactivity of the oculo-cardiac reflex when examined in the absence of depressant drugs while extreme hyper-reflex irritability was observed during anesthesia. In the other, cardiac arrest could be produced by pressure on the eyeballs when no drug had been administered. Two years later, this patient was anesthetized with nitrous oxide, cyclopropane and with ether. During the period of anesthesia, no hyperactivity of the two reflexes under consideration could be elicited.

A frank statement from clinical anesthesia at this time must confess to considerable lack of understanding of the interrelation between anesthesia and autonomic control. Definite knowledge of the influences of narcotic drugs upon reflex mechanisms and of reflex mechanisms upon the surgical patient is tremendously important. Perhaps those who establish the scientific facts in the laboratory upon normal animals may have

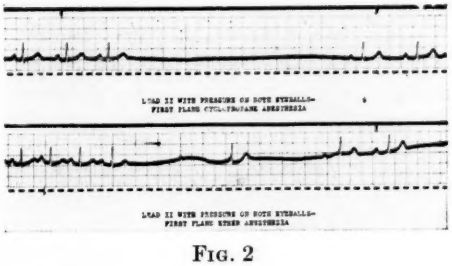


FIG. 2

to come into the hospital to help us in translating these facts and in applying them in the solution of some of our clinical problems.

COOPERATIVE EFFORT. If work in the laboratory is to come to its most glorious fruition, it must receive adequate trial and acceptance in the practice of medicine. When experiments upon animals have established a well-substantiated "hunch," why must you abandon your effort to publication in a scientific journal and the "land of the forgotten" so far as your interest is concerned? The student of basic science who follows his original concept to its ultimate test at the bedside and on the operating table will less often make mistakes in his choice of a subject for study. He can supervise the clinical tests of his work with a personal interest and economy of effort that no other individual can contribute.

In some instances research laboratories have been established by hospitals and directed by clinicians. Excellent work has been done under such circumstances. Is it not possible, however, that by this plan we may be in danger in the long future of metamorphosing excellent clinicians

into second rate basic scientists, or what may be more deplorable still, changing excellent basic scientists into second rate practitioners?

Some system ought to be workable whereby the master experimenter can rule in his own kingdom and yet not be confined like a prisoner by its boundaries. Perhaps the same may be true of the clinician. It has never been my ambition to become a physiologist or a pharmacologist. I only hope to be a clinical anesthetist and to utilize available scientific knowledge in my clinical practice. In older days, the saying was "the shoemaker should stick to his last." In years gone by, farmers indulged in the convenience of "changing work." That means that at certain seasons of the year, neighbors helped each other for a day or a few days when several men working together could more easily accomplish a task which had to be done. In what follows, I should like to suggest the value of "changing work" between the laboratory and the clinic. From personal experience, I can say that the hours I have spent as the guest of the laboratories at Wisconsin have added materially to my understanding of investigative labor and its significance to clinical anesthesia. The value of personal contact with those individuals from the laboratories who have worked with us for short periods in the hospital wards and operating rooms has been incalcuable. These contacts have not made clinicians of basic scientists nor have they made laboratory experts of anesthetists, yet each has benefited in helping the other.

It is my own belief, and that of other clinical anesthetists, that the surgical patients in our hospitals can furnish the opportunity for cooperative study by the biologic scientist through the department of anesthesiology. The anesthetized patient is not harmed by judiciously planned studies carried out upon his changing functions while he sleeps. The discovery of facts so arrived at may be of immediate benefit to him—certainly so on his return to the hospital at a later time.

Entree for the clinician to the laboratory is not difficult to establish, provided he will learn neither to lean against the unvarnished kymograph record nor to ask unnecessarily foolish questions. On the other hand, similar courtesy for the biologic scientist in the surgeon's operating room may be more difficult to secure. Such rooms are often crowded, and the occupants extremely busy and, not infrequently, working under tension.

In proportion to his skill and knowledge of clinical anesthesia, and only in that proportion, will the anesthetist enjoy the respect and confidence of the remaining members of the surgical team. Without this respect and confidence, sponsorship of the biologic scientist and his apparatus by the anesthetist can not but fail. Judicious consideration for the rights of others, added to such respect and confidence, will win approval for the presence of laboratory apparatus and even for the cardinal sin of delay in operating time. If adequate reasons are given and adequate protection of the patient is maintained, the surgeon has offered his unqualified approval.

SUMMARY

I have tried to suggest that the change in viewpoint of clinical anesthesia has been in a direction away from emphasis on drugs and apparatus and toward fundamental understanding. With fundamental understanding of those things for which the Federation of the American Societies for Experimental Biology is striving, improvements in the administration of drugs for the relief of pain are assured.

I have ventured to intimate that a cooperative relation—indulged in by laboratory worker and clinical anesthetist—the former learning a little of the operating room need and the latter a little of the scientific method, may prove mutually beneficial in promoting such understanding. I have expressed a lack of enthusiasm for the clinical anesthetist as a laboratory expert and for the latter as a clinician.

Developments in the direction suggested have so far caused clinical anesthesia to cease to look so much for its salvation in a new drug as in a better protection of the physiologic functions of the body while it is depressed by any drug which relieves pain.

With such a viewpoint and such understanding, I believe clinical anesthesia can assure the patient of reasonably safe relief from pain while it provides the surgeon with reasonably satisfactory conditions in which to accomplish his task.

REFERENCES

(1) Bigelow, H. J. Boston M. and S. J. **35:** 309, no. 16, 1846. (2) Snow, J. London Med. Gazette **42:** 1021, 1848. (3) Waters, R. M. J. A. M. A. **115:** 1687, 1940. (4) Burford, G. E. and O. R. Jones. J. A. M. A. **110:** 1092, 1938. (5) Waters, R. M. Anesthesiol. **1:** 136, 1940. (6) Gillespie, N. A. Endotracheal Anesthesia. Univ. of Wisconsin Press, 1941. (7) Mousel, L. H. and H. Essex. Anesthesiol. **2:** 272, 1941. (8) Burstein, C. L. and E. A. Rovenstine. Anesth. and Analg. **16:** 151, 1937. (9) Woodbridge, P. D. Anesth. and Analg. **12:** 161, 1933. (10) Sword, B. C. Anesth. and Analg. **9:** 108, 1930. (11) Waters, R. M. Anesth. and Analg. **18:** 74, 1939. (12) Barach, A. L. New York State J. Med. **37:** 1095, 1937. (13) Himwich, H.

E., F. A. D. Alexander and B. Lipetz. Proc. Soc. Exper. Biol. and Med. **39:** 367, 1938. (14) Metz, C. W., A. A. Wearner and A. E. Evans. South. Med. J. **32:** 34, 1939. (15) End, E. Am. J. Physiol. **120:** 712, 1937. (16) Waters, R. M. Proc. Roy. Soc. Med. **30:** 1, 1936. (17) Guedel, A. E. Anesthesiol. **6:** 13, 1940. (18) Nosworthy, M. D. Proc. Roy. Soc. Med. **34:** 479, 1941. (19) Craaford, C. On the technique of pneumonectomy in man. Tryckeri Aktiebolaget Thule, 1938. (20) Seevers, M. H., R. T. Stormont, H. R. Hathaway and R. M. Waters. J. A. M. A. **113:** 2131, 1939. (21) Seevers, M. H., R. T. Stormont and H. R. Hathaway. J. Pharmacol. and Exper. Therap. **68:** 365, 1940. (22) Beecher, H. K. J. Thoracic Surg. **10:** 202, 1940. (23) Rovenstine, E. A. and S. J. Martin. J. Indiana State Med. Assoc. **34:** 201, 1941. (24) Hand, L. V. and H. Adams. J. A. M. A. **118:** 133, 1942. (25) Meek, W. J., H. R. Hathaway and O. S. Orth. J. Pharmacol. and Exper. Therap. **61:** 240, 1937

AMINO ACIDS IN HEMOGLOBIN FORMATION

FRIEDA S. ROBSCHEIT-ROBBINS

Department of Pathology, The University of Rochester School of Medicine and Dentistry, Rochester, N. Y.

Hemoglobin formation necessitates availability of a pigment radicle, iron and a protein molecule, globin. When considering hemoglobin formation, however, one usually thinks of the pigment radicle as of greater importance, and one is less concerned with the globin portion (95 per cent) of the hemoglobin molecule. The needed pigment radicle can be manufactured by the anemic dog as demonstrated by the anemic bile fistula dog when given hemoglobin by vein. Considerable evidence has also accumulated concerning the limiting factor of iron intake for new hemoglobin production in an anemic dog whose reserve stores of iron have been depleted. In this type of anemia, iron is the most potent inorganic factor for hemoglobin production. Furthermore, low protein intake can restrict the formation of hemoglobin in anemia.

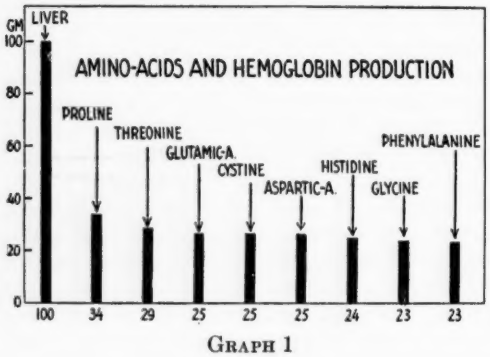
The globin portion of the hemoglobin molecule and factors which influence its formation is, therefore, deserving of careful investigation. This protein factor and its components are of present interest. While analyses of the chemical composition of globin are available, at least as concerns its amino acids[1], we still know relatively little about its manufacture within the animal organism or what happens when it is broken down in body metabolism. Dogs rendered anemic by blood removal can utilize not only native but foreign globin (horse) for the production of new dog hemoglobin. Feeding globin to the anemic dog results in a return of 30 to 40 per cent as new hemoglobin—evidence of excellent utilization of this protein. Hemoglobin itself when administered by vein to the anemic dog is quantitatively returned as new hemoglobin. This applies to sheep and goose as well as dog hemoglobin. For such purposes we would believe that this protein fraction must first be broken down to some intermediate and then rebuilt into new dog hemoglobin.

The effect of *dietary* protein on hemoglobin production has been described. Studies concerning the influence of *body protein* have indicated the efficient contribution of this protein factor to hemoglobin formation and its contained globin. To illustrate—an anemic dog during a fast will produce a considerable amount of new hemoglobin. If during this fasting period iron is administered, such an animal may produce as much as 100 gm. of new hemoglobin. Our interest in protein factors, dietary protein, tissue protein, hemoglobin, globin naturally centers on protein building stones, the amino acids. Their effect on hemoglobin production has therefore been investigated.

Previously reported experiments have given evidence that amino acids are utilized in the complex reaction related to hemoglobin building under the stimulus of along continued anemia due to blood loss. One may assume that such amino acids added to a basal ration are absorbed with the mass of amino acids derived from digestion of dietary protein. The body is able to furnish the balance of amino acids needed for building the hemoglobin molecule from diet protein, protein stores, or from protein wear and tear. In case negative results are obtained from such an experiment, one may believe that the needed supplement is either not available at the time or that the amino acid was used for more urgent demands within the organism. The perfect experiment is always the desired goal of achievement. We hoped that systematic testing of amino acids would show that one or more of these substances are of prime importance for the building of globin; however, such a hope was not realized. Imperfect experiments and indirect

approach are still the paths toward knowledge in physiology. For such experiments simplification of diet protein is desirable but no protein diet is truly simple and very few proteins are completely understood. The body can apparently break down amino acids and make other amino acids from the fragments. Schoenheimer and Rittenberg in isotope studies (N^{15} and deuterium) have demonstrated direct replacement of amino acids in proteins by dietary amino acids. Such isotopic technic reveals occurrence of rapid amino shifts among the amino acids of proteins in animals. Amino acids such as histidine, tryptophane, glutamic acid, arginine, proline and their effect on hemoglobin formation have interested various workers for years.

Considerable controversy exists as to the potency of amino acids in stimulating hemoglobin formation. Combination of tryptophane and histidine are thought to be more effective than either of these substances alone. Alcock reports a negative effect of tryptophane on hemoglobin production. Part of the existing difference of opinion may well be due to the use of different animals; dogs may vary widely from rats or rabbits in their response in hemoglobin production. The effect of a deficiency anemia produced by an exclusive milk diet may yield results at great variance with those obtained in an anemia due to loss of blood. In our own laboratory a large variety of amino acids have been tested as to their influence on hemoglobin production in dogs made anemic by blood withdrawal. Pure crystalline amino acids are added to a portion of the basal salmon bread ration to insure 100 per cent consumption. Objection may be raised to adding amino acids to a diet containing protein of unknown amino acid composition thereby giving a confused picture of little value. Such objection may be a valid one. One must remember, however, that the internal metabolism of protein is extremely complex and we are merely adding one factor to a host of others in even the simplest type of experiment. In experiments conducted at present we are eliminating this objection in tests of amino acids, as concerns their potency in hemoglobin production, by adding these amino acids to a protein-free basal ration containing crystalline vitamin accessories. The amino acids, therefore, furnish the sole source of nitrogen intake.



GRAPH 1

Analysis of globin shows fifteen amino acids as tested by Schmidt but these account for but 71 per cent of the globin protein. Included in this list are seven indicated by Rose as essential for growth. All of these have been tested individually and found no more potent than others as concerns their capacity to promote hemoglobin formation. One might also expect that one of the four amino acids present in large quantities in globin would be more potent than others. Leucine and isoleucine together account for 29 per cent of the protein globin and with lysine and histidine make up a total of 45 per cent. Experiments did not fulfill this expectation.

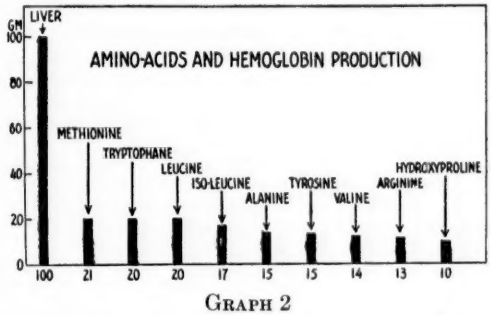
In a series of 140 experiments individual amino acids in 1 to 5 gram doses have been added to the basal salmon bread ration and fed for a two week period. The lesser 1 gm. dose per day appears to be the optimum daily intake. Larger doses (phenylalanine, glutamic acid, histidine, methionine, isoleucine, and glycine) in a few experiments rarely increase the output of hemoglobin, but smaller doses than 1 gram seem to decrease the production in some experiments. It is of interest to note that the optical isomers react in the same manner as the natural forms. Increasing the intake of the optical isomers to double does not increase the hemoglobin output. Du Vigneaud and Irish have reported that animals are able to invert "unnatural" amino acids into "natural" forms. Inversion involving complete deamination to corresponding keto-acids followed by asymmetric amination is suggested.

Graph 1 indicates the effect of a variety of amino acids on hemoglobin formation. The reaction to the standard test of liver with an average hemoglobin output of 100 grams serves as a means of comparison. The columns indicate the average net hemoglobin production resulting from feeding daily 1 to 2 grams of the amino acid indicated for a two week period. Net hemoglobin production represents the hemoglobin formation over and above that due to the basal output related to the standard basal ration. Adjustment is made for any difference in hemoglobin levels at start and end of experiment. Proline is the most effective of the amino acids tested resulting in a hemoglobin output of 34 grams, the average of five experiments. Threonine follows with a production of 29 grams of hemoglobin. The presence of this amino acid in the hemoglobin molecule has not as yet been determined. It is of interest to

note that glutamic acid, cystine and aspartic acid give a similar response—an output of 25 grams hemoglobin. Larger intakes of 3 to 5 grams do not modify the hemoglobin output. Schoenheimer and Rittenberg have suggested that glutamic and aspartic acids, dicarboxylic acids, should play the central rôle in protein metabolism. This suggestion is based on studies of administered isotopic amino acids. The concentration of the isotopic elements was found to be much higher in the dicarboxylic acids. As concerns hemoglobin formation, however, these amino acids offer little more than either cystine or histidine. Histidine with a representation of 7.6 per cent in the globin molecule is not nearly as effective as proline with but 2 per cent. The body can form glycine but it is not present in the globin molecule and one might think that additional administration would not influence the production of hemoglobin; actually, however, the average output of twelve experiments is 23 grams of hemoglobin. Phenylalanine has been tested in the natural, isomeric and dl- synthetic forms and all show a positive effect with a reaction fairly uniform for the different forms.

Graph 2 shows lesser values for hemoglobin output resulting from tests with the amino acids indicated. Methionine is somewhat less effective than cystine. Tryptophane and leucine give a similar response. One might have expected that leucine and isoleucine would show greater potency because these two amino acids together account for 29 per cent of the amino acid component of globin. Alanine, tyrosine, and valine again give similar values, 15 and 14 grams hemoglobin respectively. Arginine is but slightly less effective. It was impossible to obtain sufficient amounts of hydroxyproline for adequate testing. Two experiments only show a low net hemoglobin output. To make extra hemoglobin the dog must supply other amino acids to supplement the specific ones given. Long continued and severe anemia is a stimulus for the dog to utilize all available material to make the badly needed hemoglobin. Added amino acids may accelerate the flow of other amino acids in the direction of globin construction. If an animal can break up certain amino acids and recombine these fragments to form other amino acids and body protein, there is no reason why this same reaction can not take place in the rapid formation of the protein hemoglobin in experimental anemia.

The crucial test of the potency of amino acids for hemoglobin production is of course with an animal whose reserve protein stores are truly depleted. By reserve protein stores we may indicate material available from which the body can manufacture new hemoglobin or plasma protein or cell protein. This material may represent protein stored as a result of favorable diet, protein given up by an organ or tissue, or that derived from normal wear and tear of cells. Depletion of these stores is readily accomplished by fasting, low protein diets, and by actual protein removal by means of blood withdrawal. Simultaneous anemia and hypoproteinemia may reduce such stores to almost zero. A series of experiments is now in progress with this type of experimental animal. These dogs have been subjected not only to hemoglobin depletion but also to that of plasma proteins as well. This laboratory has been interested for several years in plasma protein regeneration and considerable data have been accumulated as to the potency of many protein substances on plasma protein production. Substances, for example, globin, which stimulate hemoglobin formation may not affect plasma protein production. The plasma protein depleted dog does not utilize globin to make new plasma



GRAPH 2

protein. The anemic dog on the other hand has little use for plasma in order to meet its hemoglobin need, while the plasma protein depleted dog uses this protein most efficiently to supply its plasma protein requirements. Further examples of contrast are casein and salmon bread, both of which are well used to produce plasma proteins and yet are relatively inert as to hemoglobin formation. Hemoglobin is a peculiar protein and it is possible that its fabrication in the body differs in many respects from that of plasma proteins and tissue proteins. The disintegration of hemoglobin may also be quite different. We know relatively little of the fate of the globin portion in the destruction and wear and tear process of the red cell.

An animal, therefore, simultaneously depleted of both proteins, hemoglobin and plasma proteins, seems an ideal testing machine. With abundant iron intake and a protein-free diet with sustained anemia due to blood withdrawal, such a dog offers excellent opportunity to study the capacity of that animal to produce new hemoglobin and new plasma protein. In this emergency of double

depletion, the dog uses available protein frugally but always produces more hemoglobin than plasma protein even when the stimulus for both is maximal. Preference is given to hemoglobin production no matter what type of protein is fed. If on the other hand, an animal is depleted of plasma protein alone and the dog can exert its energy to manufacturing new plasma protein, it can produce more plasma protein than the anemic dog can hemoglobin. A generous protein intake is of course essential. This double stress is a heavy load for the animal and it is not easy to keep such a dog otherwise physiologically normal. Food consumption is not always adequate when plasma protein levels are depleted to the desired low level. This of course means a greater loss of weight with tissue protein being added to the melange of available protein material. The anemic dog during protein fast can conserve nitrogenous precursors of urea derived from body protein breakdown and recast these substances into new hemoglobin. In contrast, the plasma protein depleted animal produces very little plasma protein from disintegration of body protein if all protein reserve stores are truly depleted. In spite of some of these experimental difficulties, it has been possible to accumulate data of interest. As a prelude to pure amino acid feeding experiments, various protein digests have been tested. These enzymatic digests of both casein and hemoglobin represent material a portion of which is composed of mixtures of amino acids. Casein digests have been studied in anemic animals, in plasma protein depleted dogs, and in experiments in dogs simultaneously depleted of both hemoglobin and plasma protein. Such animals have the usual anemic hemoglobin level of about 6 gm. per 100 cc. of blood, and a plasma protein level of approximately 4.8 per cent. This anemic and hypoproteinemic base line is kept a constant as nearly as possible by frequent blood removal and a protein-free diet. Iron is added to provide for maximum hemoglobin production. The only nitrogen intake, therefore, is that of the test substance.

TABLE 1

Casein digest: hemoglobin and plasma protein production

DAIL INTAKE (AV.)	HEMOGLOBIN (AV. PER WK.)	PLASMA PROTEIN	
		Av. per wk.	Level av.
grams	*grams*	*grams*	*per cent*
Oral—35*.....	35	20	5.1
Vein—31*.....	23	16	5.7
Control—basal.....	14	8	4.8

* Plus basal ration, Fe, vitamins.

Table 1 indicates the effect of a casein digest upon hemoglobin and plasma protein formation. This digest is an enzymatic one of casein (papain-Eli Lilly and Company) and is tested both by oral and intravenous administration. The influence of this material on both hemoglobin and plasma protein production is shown in the table. This chart represents the average production of hemoglobin and plasma protein per week computed from four similar experiments. The addition to the basal non-protein ration of 35 grams casein digest daily for a two week feeding period results in a production of 35 grams hemoglobin and 20 grams plasma protein. At the same time the base line level of plasma protein is raised from 4.8 per cent (the beginning level) to 5.1 per cent. This may be compared with the control base line indicated as the third line on the chart. The average net hemoglobin production per week, therefore, represents the difference between these two figures, 35 and 14, or 21 grams hemoglobin produced over and above that due to the basal diet alone. Similarly, the average plasma protein production is a total of 20 grams and an average net output per week of 12 grams (the difference between 20 and 8). The actual plasma protein production is slightly greater since the average difference of 0.3 per cent in level between beginning and end of the experiment can account for an approximate additional 3 grams. This same casein digest injected intravenously in a slightly lesser amount does not appear to be as potent as the oral administration. The amount introduced is optimal from a technical standpoint because larger doses produce some slight intoxication as evidenced by poorer food consumption or possible vomiting. This digest injected daily in an average dose of 31 grams was prepared with aseptic precautions. The injection was given very slowly and was continued daily for a two week period. The basal protein-free ration was fed later. The average total hemoglobin production was slightly less than that of the oral administration experiments. Plasma protein production was about equal to the oral tests if one takes into consideration the average difference in plasma protein levels of 0.6 per cent (circulating plasma volume 900 cc.). Slight toxicity of the material when given by vein can not be excluded. This casein digest may be said to have a moderate influence on the production of both hemoglobin and plasma protein in a dog depleted of both. During casein digest administration either oral or by vein, the animals were either in Nitrogen (urinary) equilibrium or in slightly positive balance. This casein digest when given to a dog depleted of plasma protein alone is extremely effective in promoting abundant new plasma protein formation.

Enzymatic (papain) digests of hemoglobin have

been tested by intraven ous administration. Oral experiments with this material are also in progress. Table 2 represents a type experiment of the series. In this experiment the digest was injected in a 30 gram dose daily for a two week period. The material was sterile and injection proceeded without any clinical symptoms of intoxication. The protein-free ration was fed following the injection. The chart describes the results obtained in the dog week by week. Other tests are similar. One point is worthy of emphasis at the beginning. No hemoglobin production occurs during the injection period but considerable formation of both hemoglobin and plasma protein is evident in the after period when injections have been terminated and the animal is again in a protein-free period. This response indicates possible intoxication produced by slightly toxic material. Experiments in anemic dogs—not depleted of plasma proteins—have given ample evidence of a definite inhibition in hemoglobin formation when intoxication exists. Apparently a disturbance of internal metabolism related to hemoglobin building is responsible for the lack of hemoglobin production. A similar course of events may occur in the dog depleted of plasma protein alone. We see, therefore, inhibition of production of both hemoglobin and plasma protein during the injection period but once this brake is released, both hemoglobin and plasma protein formation proceed as before. The actual total hemoglobin production in the after three week period is 64 grams and plasma protein 39 grams. For want of space a base line is not indicated on the table, the figures are 9 grams for hemoglobin and 7 grams per week for plasma protein. One may argue that the plasma protein level of this dog was not sufficiently reduced and, therefore, a maximal stimulus to plasma protein production did not exist. It is difficult at times to reduce plasma proteins to a desired hypoproteinemic level because a low hemoglobin figure precludes further blood removal and, therefore, a simultaneous removal of plasma proteins.

A progress report of an amino acid experiment in a dog depleted both of hemoglobin and plasma protein may be described even though it represents a negative experiment as concerns hemoglobin and plasma protein production. These studies have just been started and the experiment in question is a forerunner of a series. The animal concerned is an Eck fistula dog. The use of this type of animal for the present experiment may be subject to criticism. This particular dog was chosen because in the doubly depleted series it is by far the best reacting animal. His food consumption surpasses the others in this group, he has tolerated the double depletion of hemoglobin and plasma protein with less difficulty and for a longer time (10 mos.), and in all of the experiments has reacted as a normal animal. Similar experiments are of course being carried out in other dogs with simultaneous depletion of both hemoglobin and plasma protein. We wish to refer to this experiment as a preliminary one as it demonstrates present problems studied concerning amino acids. A mixture of amino acids containing those needed for growth requirements as reported by Rose with the exception of methionine was employed. In experiments dealing with plasma protein depletion alone cystine has been found to be a "key" amino acid for plasma protein production. Cystine, therefore, was used to replace methionine, but choline was added to the mixture. Pure crystalline amino acids[1]

TABLE 2

Hemoglobin digest—vein: hemoglobin and plasma protein production

DAILY INTAKE	HEMOGLOBIN PER WK.	PLASMA PROTEIN	
		Per wk.	Level
grams	*grams*	*grams*	*per cent*
Digest 30*.....	1	0	5.6
Digest 30*.....	2	0	5.4
Basal, Fe, vit.....	13	10	5.2
Basal, Fe, vit.....	29	17	5.2
Basal, Fe, vit.....	22	12	5.4

* Plus basal ration, Fe, vitamins.

composing the following formula were mixed with the protein-free basal ration:

	grams		*grams*
dl threonine.....	2.0	l-tryptophane.....	0.5
dl valine.....	2.0	dl phenylalanine.....	1.3
dl leucine.....	5.0	l histidine HCl.....	2.0
dl isoleucine.....	2.0	d arginine HCl.....	1.0
dl lysine HCl.....	5.0	l cystine.....	1.5
		glycine.....	5.0

The protein-free ration is composed of cornstarch, sugar, lard, vegetable oil, a suitable salt mixture, dextrin, bone-ash, baking powder and water. These ingredients are baked into a hard biscuit similar to dog biscuit. It is palatable and consumed satisfactorily for four to six week periods by most of these doubly depleted dogs. Vitamin accessories, also crystalline material, iron and choline all contained in capsules, are mixed with the food, moistened with enough water to make the diet a mush-like consistency, and the whole was spoon-fed at intervals during a morning to insure 100 per cent consumption of the amino acid mixture. Additional basal biscuit was offered later but was only partially consumed. The dog, therefore, had as sole source of nitrogen intake the amino acid mixture. This test diet was given for seven days. Thereafter the basal biscuit, vita-

[1] We are indebted to Merck & Co. Inc. for valuable amino acids.

min accessories and iron were offered for one and one-half weeks.

Table 3 indicates abstract data of this experiment. The amino acid feeding was begun during the last four days of an experimental week, therefore, the first line contains results of feeding four doses of the amino acid mixture. The remaining three doses of the total seven were administered during the first three days of the second week.

TABLE 3

Amino acid mixture: hemoglobin and plasma protein production

DAILY INTAKE	HEMOGLOBIN PER WK.	PLASMA PROTEIN	
		Per wk.	Level
	grams	*grams*	*per cent*
Amino acids*—4 doses....	2	0	4.0
Amino acids*—3 doses....	11	6	3.8
Basal biscuit†.....	8	4	3.8
Basal biscuit†.....	0	0	3.4

* Plus basal ration, Fe, vitamins.
† Plus Fe, vitamins.

Hence, the two lines indicated on table 3 actually comprise a seven day feeding period of amino acids. During the first week no hemoglobin production other than that amount removed for blood volume, cell counts, and hemoglobin determinations is evident. Plasma protein production is zero and the level of plasma protein amounts to 4 per cent. The second week with the balance of three doses of the amino acid mixture indicates a hemoglobin output of 11 grams and plasma protein output of 6 grams. The plasma protein level however has decreased to 3.8 per cent. During the first week following the amino acid feeding period while the dog was given the basal protein free ration alone, an additional 8 grams of hemoglobin was produced and 4 grams of plasma protein. The plasma protein level remained unchanged. The second week (after period) shows a zero output with a further drop of plasma protein level to 3.4 per cent. The hemoglobin level at the end of the experiment has decreased considerably from the beginning level and this difference in hemoglobin levels can account for almost the entire output of hemoglobin. The same comment applies to plasma protein production. The difference of 0.6 per cent in plasma protein levels at start and end of the experiment with a circulating plasma volume of 900 cc. accounts for nearly 6 grams of plasma protein.

This amino acid mixture which theoretically furnishes all Nitrogen essentials required by the adult dog for nitrogen balance has no influence on either hemoglobin or plasma protein production in this experiment. A negative response, however, may possibly indicate that in such a protein starved animal these amino acids may have been utilized to satisfy more urgent needs than that of hemoglobin and plasma protein and does not necessarily indicate that these amino acids or some of them are not essential for the synthesis of hemoglobin and plasma protein. One may also assume that one or more supplements which the body must contribute are not available during that particular period and synthesis fails. Amino acids are incorporated in part at least into new protein and represent components essential for protein synthesis in a depleted dog.

SIGNIFICANCE OF THE ESSENTIAL FATTY ACIDS[1,2]

GEORGE O. BURR

University of Minnesota

The term "essential fatty acids" was introduced in 1930 (13) and since that time has been used rather loosely to designate a group of unsaturated acids which will bring about a renewal of growth of rats which have reached a plateau on a low fat diet.[3] Strictly speaking only one of these is essential. If linoleic acid is added to the deficient diet growth will be renewed. Like certain essential amino acids some fatty acids may more or less completely replace each other. However, there are differences in their effects which will be described later.

The pure acids which have been found by many workers to have marked curative effects on fat

[1] Assistance in the preparation of these materials was furnished by the personnel of the Works Project Administration, Official Project no. 165–1–71–124, Subproject no. 325.

[2] This discussion will be limited to those papers which have dealt directly with the physiological effect of the essential fatty acids. Many excellent reviews of the general field of fat metabolism are available to the reader.

[3] It has been known for some time that various fats and fatty acids differ in their growth promoting properties (9, 18, 43) but some recent work indicates the possibility of a new class of essential acids (6, 8, 72, 73).

deficient rats are linoleic (9,12-octadecadienoic acid), linolenic (9,12,15-octadecatrienoic acid), and arachidonic (5,8,11,14-eicosatetrenoic acid). The last structure seems to be established now that Mowry, Brode and Brown (63) have confirmed the tentative formula given by Dolby, Nunn and Smedley-MacLean (22).

The above three acids were prepared from their recrystallized bromides and may be considered "pure"; i.e. the observed effects are due to the known acids and not to impurities. Their detailed structures are shown here for ready comparison of the changes that are made in replacing one molecule with another.

$$CH_3CH_2CH_2CH_2CH_2CH{=}CH\ CH_2CH{=}CH$$
$$14\qquad 11$$
$$CH_2CH{=}CH\ CH_2CH{=}CH\ CH_2CH_2CH_2COOH$$
$$8\qquad 5$$

arachidonic

$$CH_3CH_2CH_2CH_2CH_2CH{=}CH\ CH_2CH{=}CH$$
$$12\qquad 9$$
$$CH_2CH_2CH_2CH_2CH_2CH_2CH_2COOH$$

linoleic

$$CH_3CH_2CH{=}CH\ CH_2CH{=}CH\ CH_2CH{=}CH$$
$$15\qquad 12\qquad 9$$
$$CH_2CH_2CH_2CH_2CH_2CH_2CH_2COOH$$

linolenic

Dolby *et al.* (22) state that the above formula of arachidonic acid would readily account for its formation from linoleic since the terminal 10 carbon chains are identical in the two molecules. However, such a synthesis requires further extensive changes, including the addition of two carbons and desaturation at the 5 and 8 positions. Certainly the latter change does not commonly take place, the higher animal having a much greater tendency to saturate than to desaturate fatty acids.

All of the purified preparations that have been tested are given in table 1. Many of these have been used only once on a few animals and further experiments are to be desired. However, they are here classified as having been reported positive or negative. Some interesting conclusions regarding the utilization of fatty acids can be drawn. Acids with 2, 3, 4, and 6 double bonds can more or less completely replace one another in essential function. Linoleyl alcohol can be oxidized to linoleic acid or can replace it in another manner. Hexahydroxystearic acid, in spite of poor utilization due to very high melting point, can apparently be dehydrated to a slight extent with the synthesis of an effective unsaturated fatty acid. This is in contrast to all other oxystearic acids which are in the negative group. From an examination of the negative column it can be said that rats are unable to produce essential acids in measurable quantity by desaturation, dehydration, trans-cis rearrangement, or shift of double bonds along the carbon chain.

In addition to the pure materials there have been fed many natural oils, dehydrated castor oil, partially elaidinized mixtures, alkali conjugated linoleic and linolenic acids (13, 18, 62, 65, 86), fish oil esters, lecithin, brain lipids, buttermilk lipids, yeast lipids, leaf extracts and unsaponifiable matter. It seems clear that the effects of most seed oils may be predicted from their content of linoleic and linolenic acids. It is not so definite, however, that only two animal acids are effective. Some highly unsaturated preparations have been ineffective (buttermilk lipids, yeast oil, clupanadonic acid, and certain fish oil preparations). These may have failed because of oxidation or isomerization during processing. It seems likely that fish oil acids, other than docosahexenoic, are effective in producing growth. An impure isolinoleic acid of butter has low activity which may be due to the presence of some ordinary linoleic (18). No pure isomers of the natural acids have been found effective. The isomerization which takes place during hydrogenation, alkali treatment or elaidinization lowers the effectiveness. This brings up the question of the relative curative value of different acids based upon quantitative assays. Before this can be discussed it is necessary to describe the syndrome and the method of assay.

TABLE 1

Purified fatty acids that have been tested for activity in curing fat deficiency

References are indicated by numbers in parentheses.

POSITIVE RESPONSE	NEGATIVE RESPONSE
Linoleic	All saturated acids
Linoleyl alcohol (89, 90)	Oleic (15, 30, 86)
Linolenic (15, 57, 86, 87)	Elaidic (80, 87)
Arachidonic (19, 47, 81, 90)	12-Oleic (90)
Docosahexenoic* (46)	Erucic (13-docosenoic) (90)
Hexahydroxystearic (46)	Ricinoleic (12 - hydroxyoleic) (90)
	Linolelaidic (18)
	9,11-Linoleic (18)
	α-Eleostearic (9,11,13 - linolenic) (15)
	Dioxidostearic (46)
	Trihydroxystearic (46)
	Tetrahydroxystearic (46)
	Chaulmoogric (46, 90)
	Clupanodonic† (86) (Octadecatetrenoic)

* Not purified by crystallization but by fractional distillation of the ester.

† This preparation was found to be toxic.

Fatty Acid Deficiency and its Cure by Known Acids. Careful exclusion of fat from the diet of the rat leads to 1, development of scaly skin

and caudal necrosis; 2, marked retardation of growth (fig. 1); 3, kidney lesions and hematuria (fig. 2); 4, early death. Other more or less specific effects are histological changes in ovaries, and uterus and other tissues (Maeder); poor ovulation, reproduction and lactation in the female; male sterility; excessive water consumption; high respiratory quotients and high metabolic rates (7, 12, 13, 16, 17, 28, 29, 31, 32, 33, 55, 60, 92). Macroscopically noticeable hematuria develops late and without great regularity. The percentage of animals showing this sign varies from

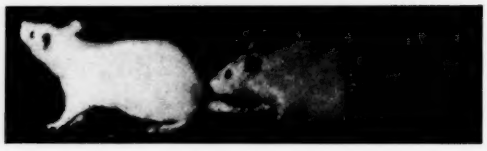


Fig. 1. Littermate sisters reared on diets with and without adequate unsaturated fatty acids. Six months of age.

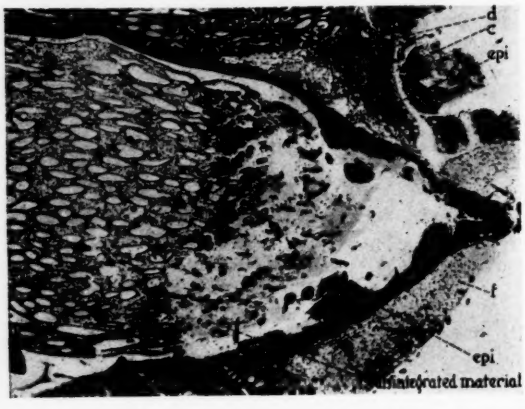


Fig. 2. Photomicrograph of a section of the kidney from test rat 3 on low-fat diet, showing necrosis, apical disintegration and calcification in the papilla; also hyperplasia of the pelvic epithelium. Hematoxylin-eosin stain. The letters indicate: d, papillary duct; c, calcification; epi, proliferated pelvic epithelium; and f, peri-renal fat. (From Borland and Jackson.)

group to group, reports ranging from 16 per cent to over 90 per cent. However, kidney lesions can be demonstrated in 100 per cent of the cases at autopsy (7). Caudal necrosis is variable and useless for assays.

Of the above effects, growth response, skin cure and ovulation of the female have been used for quantitative comparisons. Skin cure is especially useful since it distinguishes different fatty acids (14, 19, 47, 79). However, this can be used as a criterion of fat deficiency only in climates of low humidity. In fact workers at Berkeley, California, (29) report that skin effects are comparatively mild and tail necrosis has not been observed there. The great variation of degree of scaliness with humidity was studied in this laboratory statistically throughout the year and in chambers with controlled humidity. The changes in skin are shown photographically in figure 3

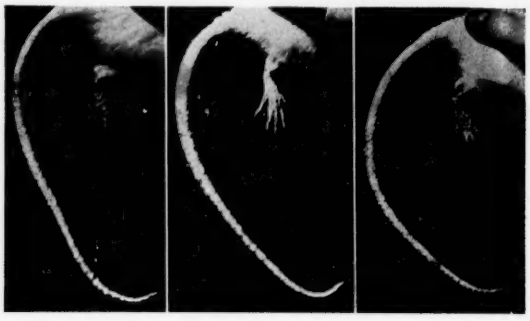


Fig. 3. Effect of humidity on scaliness. (From Brown and Burr, 1936.) Left, low fat rat from colony. Middle, same animal after 48 hours at 97 per cent relative humidity. Right, same animal after three weeks at 50 per cent relative humidity.

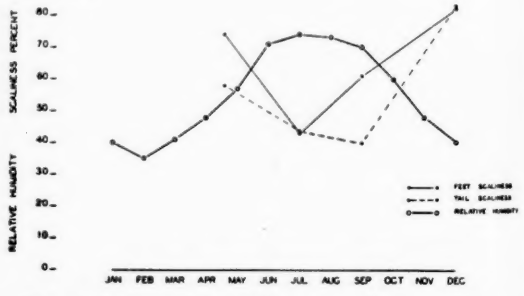


Fig. 4. Effect of humidity on scaliness. Average scaliness expressed as per cent of the maximum possible plotted against average colony humidity.

and relative scaliness compared with humidity in figure 4.

The usual procedure for the assay of a fatty acid follows. Young rats are reared on a diet of purified casein, sucrose, and salts supplemented by all known vitamins. In the early work some confusion arose due to the fact that inadequate amounts of the B-complex were used by some workers (16). To produce uncomplicated fatty acid deficiency ether extracted yeast has been widely used. If the rats do not grow rapidly at first some other deficiency should be suspected. Diets of very low fat content have been produced for special work (12, 54, 61). However, it is now known that fatty acid deficiency occurs with fairly impure diets. Crude casein, unextracted yeast, potato starch and skim milk may be used

with only a slight delay in produced symptoms (11) (see fig. 5). Many commercially canned dog foods have been found to be deficient in linoleic acid[4].

The time of appearance of symptoms, and their severity, the maximum weight attained, as well as the longevity of the rat depends to a large degree on the diet of the mother and the freedom from linoleic acid of the basal ration (18). The diet of the mother has a large effect just as in the case of vitamin A studies.

When the animals have reached a growth plateau and scaly skin has developed the curative doses are fed and the effects on weight and skin noted after 4 to 6 weeks or longer. In this laboratory an arbitrary rating of scale on the hind feet, scale and roughness of tail, and dandruff in the back hair (0 to 3) is used to give a range from zero to 9 for maximum scaly condition. By practice reliable results can be obtained. Hume *et al.* (47) have used the healing of the dorsal surface of the hind feet and ankles as the measure of skin effects. They quite rightly emphasize the importance of the skin measurements.

TABLE 2

Curative effects of fatty acids

ACID	GROWTH EFFECT	SKIN EFFECT	WATER CONSUMPTION
Linoleic	+	+	Normal
Arachidonic	+	+	Normal
Linolenic	+	−	High
Linusic	+	−	
Docosahexenoic	+	−	
Esters of cod liver oil	+	−	High

By carefully comparing the growth and skin responses it has been found that fatty acids differ in their effects. Not all of those which produce growth will also clear the skin. However, no acid has been found which clears the skin without producing growth. This differential effect was first noticed with cod liver oil, which brought about renewed growth without commensurate healing of the scaly skin (14, 79). Subsequent work has extended the observations to specific fatty acids so that they may be classified in this regard. The earlier statement by us that the cure of growth is always accompanied by cure of skin does not hold. The differences in table 2 are not absolute. If high doses of linolenic acid are fed daily there is some amelioration of the scaly skin (19). Linoleic acid is equal to or slightly better than arachidonic acid in this regard. This superiority of linoleic acid becomes pronounced when low levels are fed (19, 47) and arachidonic acid shows some resemblance to the higher acids of fish oils. The effects of these acids upon water consumption parallel their effects upon the skin; i.e., if the skin is cured the water consumption is reduced to normal. Otherwise, not. Unpublished data from this laboratory give the following daily water consumption after "cures" in one experiment: Esters of cod liver oil 18.6 ml.; ethyl linolenate 23.5 ml.; ethyl linolate 11.2 ml.; and methyl arachidonate 9.3 ml.

Quantitative comparisons of the growth responses to purified acids are not in close agreement. These discrepancies may easily be due to the different methods of testing. Turpeinen (90), using the method of minimum dose required to give maximum response, concluded that arachidonic acid is at least three times as effective as linoleic. However, the lower effect of linoleic acid in this type of experiment may be due to its much greater storage in tissues, giving a lower initial response but an equal overall effect. The lowest level fed by him (33 mg. daily) is too high for the measurement of intermediate growth rates which is desirable in assay work. Hume *et al.* (47) came to the conclusion that arachidonic acid is about twice as effective as linoleic when fed at low levels for 35 days. However, their results are not entirely regular in successive experiments. In one test the animals receiving linoleic acid grew more rapidly. Our own tests (19) tend to give arachidonic acid a somewhat greater growth effect at first. Without further studies an exact curative ratio for these two acids can not be given. As a general statement it may be said that arachidonic, linoleic, linolenic, docosahexenoic and the higher esters of cod liver oil are all very effective, with arachidonic possibly leading the list. Before leaving the question of relative values of fatty acids a word should be said about the search for natural isomers of linoleic and linolenic acids. Due to the fact that on bromination of linoleic acid only 50 per cent of the expected tetrabromostearic is precipitated, the terms α- and β-linoleic acid are in the literature to designate what was thought to be two natural isomers occurring in a 1:1 ratio. There is much evidence now, however, that there is only one linoleic acid in most plant fats (48). However, on debromination of either linoleic or linolenic bromides some changed materials are formed which can be distinguished by low temperature crystallization (58). Low temperatures can also be used for the direct separation of linoleic and linolenic acids. We have found that acids prepared from oils directly by freezing have essentially the same curative effects as those made by debromination of solid bromides (unpublished material with J. B. Brown). However, if the

[4] Private communication from C. A. Elvehjem. Paper given in Detroit, September 1940.

liquid tetrabromides are debrominated the resulting preparation is not so good (18). These preparations usually have low iodine numbers indicating undesirable changes. There is no evidence for the existence in common seed fats of a biologically inactive form of linoleic or linolenic acid.

The Daily Requirement of the Rat and Other Species for Linoleic Acid. No absolute values can be given for the optimum intake of linoleic acid. In the ordinary curative experiment no very definite response is seen with daily doses of less than 5 mgm. of either linoleic or arachidonic. However, 10 to 15 mgm. daily of linoleic gives a good measurable response in 4 to 6 weeks (19, 47). The maximum growth rate in curative experiments has been attained with the following daily doses of linoleic acid: 60 mgm. (13); 100 mgm. (90); 20 mgm. (57); 42 mgm. (46, 47); 40 mgm. (19). Martin (57) has conducted the most extensive experiments on linoleic acid from which he concluded that 20 mgm. daily gave optimum growth. After the rats had reached a plateau on the lower doses no renewed growth resulted from increasing the methyl linolate ten times. Undoubtedly the methods of testing account for the differences in results from several laboratories. When fed as a prophylactic dose throughout the growth period of young rats, 20 mgm. of linoleic acid is enough to produce an apparently normal rat. Animals free from any symptoms of fatty acid deficiency have been produced by the daily addition of 10 drops of lard (20 mgm. linoleic acid) or 23 mgm. of methyl linolate (12, 54). MacKenzie *et al.*, using a rigidly purified diet, found that the latter amount was sufficient for satisfactory growth and reproduction. The females compared favorably with stock rats over a period of eleven months whereas the males were some what smaller. This carefully conducted experiment lends strong evidence to the view that linoleic acid alone is sufficient.

It should be pointed out that these low fat diets will not produce the largest possible rats. Hoagland and Snider (43) conclude that 5 per cent of lard, oleo oil or hydrogenated fat is definitely too little. Diets containing 20 to 50 per cent fat give much larger animals. This means that fats are excellent food for growth; not that they are supplying a new unidentified factor.

The requirement for linoleic acid depends upon the composition of the diet. If the carbohydrate is replaced by a fat free from linoleic acid the growth of the rat is greatly retarded and early death ensues (29, 80) (fig. 5). These animals are cured by feeding small amounts of linoleic acid (50 mgm. daily) or corn oil (1 per cent of diet). They also resume growth when changed back to a high carbohydrate diet. Using purified elaidin as the dietary fat Sinclair finds that 20 mgm. of corn oil is entirely too little for a cure. This would indicate that the requirement for linoleic acid is greater with high fat diets or that some synthesis of linoleic acid takes place on high carbohydrate diets. There is as yet no direct evidence for this synthesis. Tissue analyses indicate that the content of linoleic acid is directly dependent upon the amount of this acid in the diet (1, 23, 52). Ellis and Hankins found that in pigs the total body linoleic acid was about one-half that which could be obtained from the low-fat diet used (fig. 6). Schonheimer (77) points out that "in contrast to all other fatty acids investigated so far linoleic and linolenic acids do not "take up" deuterium from the heavy water of the body fluids. These highly unsaturated

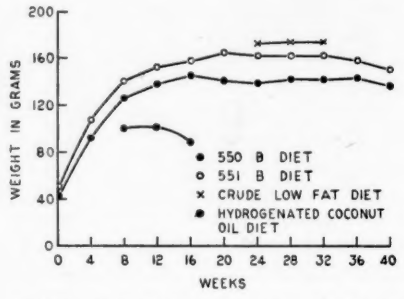


Fig. 5. Effect of diet on growth of fat deficient rats. Diet 550-B is highly purified. 551-B contains crude casein and unextracted yeast. The crude low fat diet contained skim milk and potato starch. Poorest growth and early death occurred on the diet in which hydrogenated coconut oil replaced sucrose. (Unpublished data of W. R. Brown and G. O. Burr.)

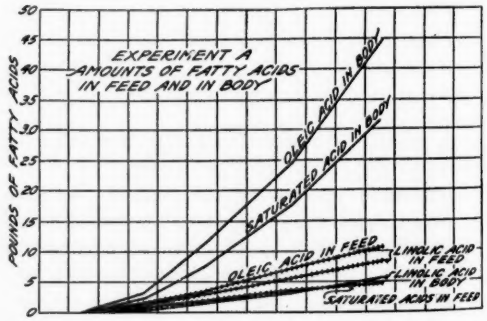

POUNDS OF FATTY ACIDS
EXPERIMENT A
AMOUNTS OF FATTY ACIDS IN FEED AND IN BODY
OLEIC ACID IN BODY
SATURATED ACID IN BODY
OLEIC ACID IN FEED
LINOLIC ACID IN FEED
LINOLIC ACID IN BODY
SATURATED ACIDS IN FEED

Fig. 6. Total fatty acids synthesized by pigs on a low-fat diet. In 230-pound pigs there had been an accumulation of 34 pounds of synthetic oleic and 28 pounds of synthetic saturated acids. Linoleic acid is constantly used leaving less stored in the body than was consumed. (From Ellis and Hankins, 1925.)

acids must have been derived directly from the diet." These results point to the conclusion that high fat diets demand more linoleic acid than do high carbohydrate diets. A choice between the alternatives of higher demand or less synthesis must await further experiments.

Very few experiments on essential fatty acid deficiency have been done with other species. In studies with cows it has been found that an appreciable amount (4 per cent or more) of dietary fat is necessary for optimum milk and fat secretion (36, 59). Although the iodine number of the blood lipids falls with the use of low fat diets, there is no clear evidence that linoleic acid itself is involved.

It has also been found that young calves do not thrive on a low fat diet composed of skim milk, beet pulp, starch, cerelose and cod liver oil (38). The fat content of the blood of these animals fell to a very low level and normal conditions were obtained by the addition of fat to the diet. However, no proof of the requirement for linoleic acid was given.

Day-old White Leghorn chicks were fed over a period of 14 weeks an extracted mash containing less than 0.1 per cent extractable fat (69). When compared with birds on the normal mash (4.1 per cent fat) they weighed slightly less (but not significantly) and the iodine numbers of their depot fats were much lower. The authors conclude that there were no signs of fatty acid deficiency but it must be remembered that the mash contained 47 per cent corn and that corn starch contains enough unextractable linoleic acid to prevent severe deficiency symptoms in rats (12, 30).

In a series of papers Ellis and co-workers (23, 24, 25, 26) report that the most striking effect of feeding low fat diets to hogs is the reduction of the linoleic acid content of the body fat. Diets containing as little as 0.8 per cent extractable fat were fed and the linoleic acid content of the lard fell as low as 1.2 per cent as compared with 7 to 15 per cent in ordinary lards. However, they do not report evidence of any deficiency symptoms.

It has long been known that dogs are especially susceptible to eczema and in recent years many people have been using some unsaturated fat to aid in clearing the skin. Experiments with dogs are now in progress at Minnesota[5] which show that puppies reared on a low fat diet develop scaly skin which is curable with lard or other fats. The iodine number of the blood lipids drops about 25 per cent under these conditions and rises as the cure takes place. Dogs seem to be readily susceptible to linoleic acid deficiency.

Very little is known regarding the requirements of man for unsaturated fatty acids. Van Gröer (91) maintained two infants for several months on a diet extremely low in fat. The growth was fairly good but one child developed a generalized eczema. Of three infants maintained on extremely low fat diets by Holt and associates (45), one developed a marked eczema. In two infants maintained at the University of Minnesota Hospital for 10 weeks on a diet extremely low in fat there was a moderate decrease in the iodine number of the serum lipids.

The above findings led to an experiment with one adult human subject maintained for six months on a diet limited to sucrose, potato starch, special skim milk, orange juice, salts, vitamins and flavoring matter (11).. The subject felt no harmful effects although young rats raised on the same diet developed typical fat deficiency symptoms. The subject began the experiment with much stored fat and no great effects should be expected in such a short period. In spite of these limitations, however, there was a change in respiratory metabolism similar to that found in fat-deficient rats and a marked fall in the arachidonic and linoleic acid contents of the serum lipids. Each fell to about 50 per cent of the starting value.

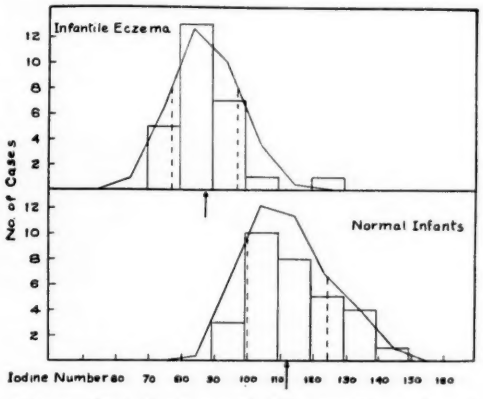


Fig. 7. Distribution of iodine numbers of serum fatty acids in normal and eczematous children under 2 years of age. (Drawn from data of A. E. Hansen.)

When it was found that scaly skin of rats was accompanied by low iodine number of serum lipids (40), similar studies were begun by Hansen and coworkers on eczematous human subjects (39, 41). There is a distinctly lower content of arachidonic acid (polybromides) and linoleic acid (tetrabromides) in the lipids of eczematous patients (10). The iodine number is usually lower and in the case of infants with uncomplicated eczema the average difference is about 25 per cent. In

[5] A. E. Hansen and H. F. Wiese.

figure 7 are summarized the values found at the University of Minnesota Hospital over a period of years (41). In a similar study by Finnerud, Kesler and Wiese (35) with hospitalized human adults 50 per cent had subnormal blood lipid iodine numbers. In all of the cases thus far studied it has been found that a large percentage of those having eczema accompanied by low blood iodine numbers respond to the addition of fats like lard or corn oil. With the clearing of the skin there is a rise in lipid iodine numbers accompanied by a rise in linoleic acid but not in arachidonic. This relation of eczema to unsaturated lipids has been found by some other workers (20, 34) but not by all (37, 88). However, the evidence seems to be accumulating in favor of the view that many eczemas are specifically related to a deficiency of unsaturated fatty acids due either to a low intake or to an abnormally high requirement by these subjects. The response of a case is illustrated in figure 8. This patient, 5 months of age, was kept in the hospital with no change of local treatment. No tar was applied. The diet was the same except

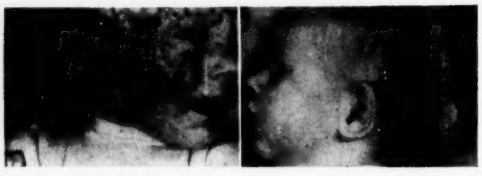


Fig. 8. Child treated with 2 to 3 teaspoons of lard daily for one month. Age 5 months. (Courtesy of Dr. A. E. Hansen.)

for the addition of 2 to 3 teaspoons of lard daily for one month. The change is very striking.

Other Physiological Relationships. In addition to the effects already discussed there have been found in the last few years numerous interesting relationships between dietary fats and other physiological functions. These will be mentioned in order of discovery.

Vitamin B_6 and essential fatty acids. The study of this relationship seems to have had its beginning in a statement by Hogan and Richardson (44) that a dermatitis similar to the vitamin B_6 syndrome could be cured by wheat germ oil. This led Birch and György (4) to find that fat had a strong sparing action on B_6. Further studies (5) showed that fat added to the diet greatly delayed or completely prevented the dermatitis even at time of death. With 20 per cent lard in the diet acrodynia was mild or entirely lacking. It was concluded that the protective action was likely due to linoleic acid. In 1936 Bender *et al.*[3] noted that the vitamin B dermatitis was delayed and less severe with rations containing 10 per cent instead of 3 per cent Crisco. Later (84) they point out that 3 per cent Crisco does not significantly decrease the incidence of acrodynia as compared with fat-free diets whereas 10 per cent delays it and 20 per cent markedly reduces it. The other fats and fatty acids studied prevent or delay the symptoms in proportion to their unsaturation.

In 1938 Salmon (70) found that vitamin B_6 and essential fatty acids supplement each other in the cure of dermatitis. Plant oils were effective in proportion to their unsaturation except that linolenic acid is not as effective as linoleic. Cod liver oil is ineffective.

In an extensive series of tests by Steenbock and coworkers (65, 74) in which many oils and foods were tested for their antiacrodynic action similar results were obtained. Linseed oil is less effective than corn oil; cod liver oil is ineffective, and an examination of their data shows that the other oils fall in the order of their estimated linoleic acid content. Schneider (75) also showed that the anti-dermatitis potency of butter fat falls with development of rancidity.

The finding by Schneider, Steenbock and Platz (76) that fatty acid deficiency can be cured by a rice bran concentrate has not yet been confirmed by other workers.

Richardson, Hogan and Itschner (68) have extended these observations in pointing out that linoleic acid is more effective than arachidonic. They emphasize again, however, that fatty acids do not replace B_6 but delay the onset of skin symptoms.

The only worker who has failed to find any "sparing action" of fatty acids on pyridoxine deficiency is Emerson (27) who found no ameliorating effect of 80 mgm. ethyl linolate daily on the florid dermatitis of rats receiving 5 μg daily of pyridoxine. She attributes the results of her experiments to the use of pure vitamins plus liver filtrate, but other workers have used pure vitamins and extracts. However, her rats did not grow very well indicating the possibility of another deficiency which might affect the skin (16). A further difference may arise from the higher humidity of her laboratory making skin observations less reliable.

A new effect of pyridoxine deficiency, a convulsive syndrome in suckling young, has just been described (21). No marked sparing effect of corn oil could be demonstrated although the survival period was slightly increased in some animals. It seems clear that the so-called pyridoxine sparing action of linoleic acid is largely one of alleviation of the acrodynia. Even 75 per cent of fat in a diet will not prevent death from B_6 deficiency (5) although the animals die without having developed severe skin symptoms.

Furthermore the different fatty acids fall into

precisely the same order as they do in curing uncomplicated scaly skin: linoleic, most active; arachidonic, intermediate; linolenic and higher fish oils, least effective.

Pantothenic acid. Richardson and Hogan (67) have pointed out that lack of pantothenic acid produces a dermatitis and that it is required along with pyridoxine for a complete skin cure. Recently (68, 71) the two vitamins have been studied in relation to each other and the essential fatty acids. Salmon concluded that all three were required for complete skin cure. Linoleic acid has a measurable effect and large doses decrease both the severity and incidence of pantothenic acid dermatitis. Arachidonic acid is less effective.

Egg white injury or biotin deficiency. MacKay and Barnes (53) found that if the Crisco in the diet was replaced by corn oil the effects of raw dried egg white were much diminished. The animals grew more rapidly and dermatitis was slight. If additional B_6 was also administered the skin symptoms almost completely disappeared.

Riboflavin. Mannering, Lipton and Elvehjem (56) have shown that high fat diets increase the requirement for riboflavin. This, like the thiamin sparing action of fats, has not been related to the essential fatty acids.

Carotene and vitamin A. Sumner and Sumner (83) have recently found that the unsaturated fatty acids enter into a coupled reaction with carotene oxidase in the bleaching of carotene. Linseed oil is more active than linoleic acid (82). It is generally recognized that vitamin A destruction by oils *in vitro* is due to peroxides and other intermediates of rancidity (49, 66). However, Lease and Steenbock (50) found no effect of fresh or rancid lard or of choline on the rate of depletion of liver vitamin A and further work showed no effect of composition of oil on carotene utilization (51). Sherman (78) has recently reported an antagonistic effect of linoleic acid toward carotene which may be an oxidation effect.

The above work has an interesting bearing on some results obtained in this laboratory. Figure 9 shows the poor growth when corn oil is homogenized into skim milk with a limited vitamin A supply. Addition of cod liver oil stimulated growth at once. This experiment has now been repeated with solid diets and rats with very limited stores of vitamin A. The addition of corn oil to the diet depleted the stores of vitamin A more rapidly than did lard. Two explanations of this are: 1, lard carries traces of vitamin A which can not be demonstrated, or 2, ingestion of corn oil increases the demand for A. This may be the explanation of the very harmful effects of unsaturated fats on calves (38).

Fat transport and utilization. Hevesy and Smedley-MacLean (42) used radioactive phosphorus to measure the synthesis of phospholipids by fat-deficient rats. They found the phosphorus turn over in liver and kidney to be the same for both deficient and cured rats. But in the muscle of the deficient rats there was a much more rapid exchange of the phosphorus.

The opposite result was obtained for intestinal mucosa by the use of spectroscopically active fatty acids in tagging experiments (2). Although there was no difference in the rate of absorption of fat by fat-deficient rats, there was a considerable drop in the rate of incorporation of labeled acids in the mucosal phospholipids.

The lipotropic action of corn oil has recently been demonstrated by Engel.[6] Using diets deficient in linoleic acid and choline it was found that choline alone did not reduce the fat content

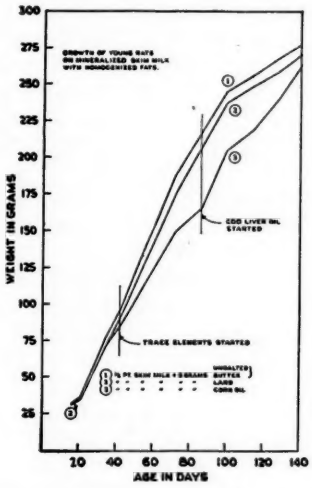


Fig. 9. Increased requirement for vitamin A in the presence of large amounts of a highly unsaturated oil (corn oil).

of the livers. The marked effect of 0.1 cc. of corn oil is presumably due to its linoleic acid.

The higher fatty acids appearing in tissues after feeding curative doses of unsaturated acids have been reported in a brief communication (64).

Conclusions. I hope the present status of the work with the essential fatty acids has been given completely enough so that you can draw your own conclusions as to their significance. Most significant to me is the increasing number of reported interrelations between the unsaturated acids and other essentials. Knowledge of the structure of the acids makes possible interesting speculation as to how they replace each other either fully or partially, but no direct information is yet avail-

[6] Private communication from R. W. Engel.

able as to the exact mechanism of their action in preventing or curing the many abnormalities associated with their lack. Undoubtedly the unsaturated acids function primarily as essential building stones in cell structures and in the mobile lipids. It would not be surprising to find numerous ill effects on domestic live stock and man of the present trend toward high carbohydrate diets containing small amounts of highly saturated fats.

Linoleic acid is especially important to the health of the skin and if plentiful in the diet will "spare" those vitamins that also affect the skin. Two or more superimposed deficiencies are worse than one but this does not mean that the vitamins are controlling fat metabolism (5) or vice versa. Because skin effects are readily noticeable they have thus far been emphasized. But when it is recalled that kidneys and other organs of all fat deficient rats also show changes it seems likely that many tissues will eventually be found to show the adverse effects of vitamin deficiencies in animals eating limited amounts of linoleic acid. This common food stuff should be plentifully supplied to avoid any such complications.

REFERENCES

(1) Banks, A., T. P. Hilditch and E. C. Jones. Biochem. J. **27:** 1375, 1933. (2) Barnes, R. H., E. S. Miller and G. O. Burr. J. Biol. Chem. **140:** 773, 1941. (3) Bender, R. C., S. Ansbacher, G. E. Flanigan and G. C. Supplee. J. Nutrition **11:** 391, 1936. (4) Birch, T. W. and P. György. Biochem. J. **30:** 304, 1936. (5) Birch, T. W. J. Biol. Chem. **124:** 775, 1938. (6) Boer, J. and B. C. P. Jansen. Voeding, June 15, no. 2, 1941. (7) Borland, V. G. and C. M. Jackson. Arch. Path. **11:** 687, 1931. (8) Boutwell, R. K., R. P. Geger, C. A. Elvehjem and E. B. Hart. J. Dairy Sci. **24:** 1027, 1941. (9) Brown, W. R. and G. O. Burr. Proc. Am. Soc. Biol. Chem. **8:** xvi, 1936. (10) Brown, W. R. and A. E. Hansen. Proc. Soc. Exper. Biol. and Med. **36:** 113, 1937. (11) Brown, W. R., A. E. Hansen, G. O. Burr and I. McQuarrie. J. Nutrition **16:** 511, 1938. (12) Burr, G. O. and M. M. Burr. J. Biol. Chem. **82:** 345, 1929. (13) Burr, G. O. and M. M. Burr. J. Biol. Chem. **86:** 587, 1930. (14) Burr, G. O., M. M. Burr and W. R. Brown. Proc. Soc. Exper. Biol. and Med. **28:** 905, 1931. (15) Burr, G. O., M. M. Burr and E. S. Miller. J. Biol. Chem. **97:** 1, 1932. (16) Burr, G. O. and W. R. Brown. Proc. Soc. Exper. Biol. and Med. **30:** 1349, 1933. (17) Burr, G. O. and A. J. Beber. J. Nutrition **14:** 553, 1937. (18) Burr, G. O. Chemistry and medicine. Univ. Minnesota Press, p. 101, 1940. (19) Burr, G. O., J. B. Brown, J. P. Kass and W. O. Lundberg. Proc. Soc. Exper. Biol. and Med. **44:** 242, 1940. (20) Cornbleet, T. Arch. Dermat. and Syph. **31:** 224, 1935. (21) Daniel, E. P., O. L. Kline and C. D. Tolle. J. Nutrition **23:** 205, 1942. (22) Dolby, D. E., L. C. A. Nunn and I. Smedley-MacLean. Biochem. J. **34:** 1422, 1940. (23) Ellis, N. R. and O. G. Hankins. J. Biol. Chem. **66:** 101, 1925. (24) Ellis, N. R. and H. S. Isbell. J. Biol. Chem. **69:** 219, 1926. (25) Ellis, N. R. and H. S. Isbell. J. Biol. Chem. **69:** 239, 1926. (26) Ellis, N. R. and J. H. Zeller. J. Biol. Chem. **89:** 185, 1930. (27) Emerson, G. Proc. Soc. Exper. Biol. and Med. **47:** 445, 1941. (28) Evans, H. M. and G. O. Burr. Proc. Soc. Exper. Biol. and Med. **25:** 390, 1928. (29) Evans, H. M. and S. Lepkovsky. J. Biol. Chem. **96:** 157, 1932. (30) Evans, H. M. and S. Lepkovsky. J. Biol. Chem. **96:** 143, 1932. (31) Evans, H. M., S. Lepkovsky and E. A. Murphy. J. Biol. Chem. **106:** 445, 1934. (32) Evans, H. M., S. Lepkovsky and E. A. Murphy. J. Biol. Chem. **106:** 441, 1934. (33) Evans, H. M., S. Lepkovsky and E. A. Murphy. J. Biol. Chem. **106:** 431, 1934. (34) Faber, H. K. and D. B. Roberts. J. Pediatrics **6:** 490, 1935. (35) Finnerud, C. W., R. L. Kesler and H. F. Wiese. Arch. Dermat. and Syph. **44:** 849, 1941. (36) Gibson, G. and C. F. Huffman. Michigan Agric. Exper. Sta. Quart. Bul. **244:** 1939. (37) Ginsberg, J. E., C. Bernstein, Jr. and L. V. Iob. Arch. Dermat. and Syph. **36:** 1033, 1937. (38) Gullickson, T. W., F. C. Fountaine and J. B. Fitch. J. Dairy Sci. **25:** 117, 1942. (39) Hansen, A. E. Proc. Soc. Exper. Biol. and Med. **30:** 1198, 1933. (40) Hansen, A. E. and G. O. Burr. Proc. Soc. Exper. Biol. and Med. **30:** 1201, 1933. (41) Hansen, A. E. Am. J. Dis. Child. **53:** 933, 1937. (42) Hevesy, G. C. and I. Smedley-MacLean. Biochem. J. **34:** 903, 1940. (43) Hoagland, R. and G. R. Snider. U. S. Dept. Agric. Tech. Bul. no. 725. (44) Hogan, A. G. and L. R. Richardson. Nature **136:** 186, 1935. (45) Holt, L. E., Jr., H. C. Tidwell, C. M. Kirk, D. M. Cross and S. Neale. J. Pediatrics **6:** 427, 1935. (46) Hume, E. M., L. C. A. Nunn, I. Smedley-MacLean and H. H. Smith. Biochem. J. **32:** 2162, 1938. (47) Hume, E. M., L. C. A. Nunn, I. Smedley-MacLean and H. H. Smith. Biochem. J. **34:** 879, 1940. (48) Kass, J. P., W. O. Lundberg and G. O. Burr. Oil and Soap **17:** 50, 1940. (49) Lease, E. J., J. G. Lease, J. Weber and H. Steenbock. J. Nutrition **16:** 571, 1938. (50) Lease, E. J., and H. Steenbock. J. Nutrition **17:** 85, 1939. (51) Lease, E. J., J. G. Lease, H. Steenbock and C. A. Baumann. J. Nutrition **17:** 91, 1939. (52) Longenecker, H. E. J. Biol. Chem. **128:** 645, 1939. (53) MacKay, E. M. and R. H. Barnes. Proc. Soc. Exper. Biol. and Med. **46:** 353, 1941. (54) MacKenzie, C. G., J. B. MacKenzie and E. V. McCollum.

Biochem. J. **33:** 935, 1939. (55) MAEDER, E. C. Anat. Rec. **70:** 73, 1937. (56) MANNERING, G. J., M. A. LIPTON AND C. A. ELVEHJEM. Proc. Soc. Exper. Biol. and Med. **46:** 100, 1941. (57) MARTIN G. J. J. Nutrition **17:** 127, 1939. (58) MATTHEWS, N. L., W. R. BRODE AND J. B. BROWN. J. Am. Chem. Soc. **63:** 1064, 1941. (59) MAYNARD, L. A., K. E. GARDNER AND A. HODSON. Cornell University Agric. Expt. Sta. Bull. 722, 1939. (60) MCAMIS, A. J., W. E. ANDERSON AND L. B. MENDEL. J. Biol. Chem. **82:** 247, 1929. (61) MCKIBBIN, J. M., J. J. OLESON, C. A. ELVEHJEM AND E. B. HART. Proc. Soc. Exper. Biol. and Med. **41:** 25, 1939. (62) MOORE, T. Biochem. J. **31:** 138, 1937. (63) MOWRY, D. T., W. R. BRODE AND J. B. BROWN. J. Biol. Chem. **142:** 679, 1942. (64) NUNN, L. C. A. AND I. SMEDLEY-MACLEAN. Biochem. J. **32:** 2178, 1938. (65) QUACKENBUSH, F. W., B. R. PLATZ AND H. STEENBOCK. J. Nutrition **17:** 115, 1939 (66) QUACKENBUSH, F. W., R. P. COX AND H. STEENBOCK. Proc. Am. Soc. Biol. Chem., J. Biol. Chem. **140:** civ, 1941. (67) RICHARDSON, L. R. AND A. G. HOGAN. Proc. Soc. Exper. Biol. and Med. **44:** 583, 1940. (68) RICHARDSON, L. R., A. G. HOGAN AND K. F. ITSCHNER. Univ. of Missouri Agric. Exper. Sta. Research Bull. 333, 1941. (69) RUSSELL, W. C., M. W. TAYLOR AND L. J. POLSKIN. J. Nutrition **19:** 555, 1940. (70) SALMON, W. D. Proc. Am. Soc. Biol. Chem., J. Biol. Chem. **123:** civ, 1938. (71) SALMON, W. D. Proc. Am. Soc. Biol. Chem., J. Biol. Chem. **140:** cix, 1941. (72) SCHANTZ, E. J., R. K. BOUTWELL, C. A. ELVEHJEM AND E. B. HART. J. Dairy Sci. **23:** 1205, 1940. (73) SCHANTZ, E. J., C. A. ELVEHJEM AND E. B. HART. J. Dairy Sci. **23:** 181, 1940. (74) SCHNEIDER, H. A., J. K. ASCHAM, B. R. PLATZ AND H. STEENBOCK. J. Nutrition **18:** 99, 1939. (75) SCHNEIDER, H. A. Proc. Soc. Exper. Biol. and Med. **44:** 266, 1940. (76) SCHNEIDER, H., H. STEENBOCK AND B. R. PLATZ. J. Biol. Chem. **132:** 539, 1940. (77) SCHOENHEIMER, R. AND D. RITTENBERG. Physiol. Reviews **20:** 218, 1940. (78) SHERMAN, W. C. J. Nutrition **22:** 153, 1941. (79) SINCLAIR, R. G. Proc. Soc. Exper. Biol. and Med. **27:** 1059, 1930. (80) SINCLAIR, R. G. J. Nutrition **19:** 131, 1940. (81) SMEDLEY-MACLEAN, I. AND L. C. A. NUNN. Biochem. J. **34:** 884, 1940. (82) SÜLLMAN, H. Helv. Chim. Acta **24:** 646, 1941. (83) SUMNER, J. B. AND R. J. SUMNER. J. Biol. Chem. **134:** 531, 1940. (84) SUPPLEE, G. C. R. C. BENDER AND O. J. KAHLENBERG. J. Nutrition **20:** 109, 1940. (85) TANGE, U. Sci. Papers Inst. Phys. and Chem. Res. **20:** 13, 1932. (86) TANGE, U. Sci. Papers Inst. Phys. and Chem. Res. **20:** 13, 1933. (87) TANGE, U. Sci. Papers Inst. Phys. Chem. Research (Tokyo) **36:** 482, 1939. (88) TAUB, S. J. AND S. J. ZAKON. J. A. M. A. **105:** 1675, 1935. (89) TURPEINEN, O. Proc. Soc. Exper. Biol. and Med. **37:** 37, 1937. (90) TURPEINEN, O. J. Nutrition **15:** 351, 1938. (91) VON GRÖER, F. Biochem. Ztschr. **97:** 311, 1919. (92) WESSON, L. G. AND G. O. BURR. J. Biol. Chem. **91:** 525, 1931.

American Physiological Society

Symposium on The Mechanism of Secretion

A. C. Ivy, Chairman

April 4, 1942

SOME MORPHOLOGICAL ASPECTS OF THE SECRETORY PROCESS

ALDEN B. DAWSON

The Biological Laboratories, Harvard University

While the morphologist and physiologist have a common interest, the complete understanding of the secretory process, their methods of approach to such a problem and the emphasis of their interest are naturally different. The morphologist is concerned primarily with the microscopically-visible components involved in the elaboration of the secretory product; the physiologist with the mode of control of glandular activity, the chemical composition, the manner and rate of release of the product, and the physiological effects produced upon release. Both are studying *secretion* but may not even have the same mental concept when they use the term. Secretion to the morphologist usually relates to the mode of intracellular origin, elaboration and accumulation of the product, while secretion to the physiologist has reference chiefly to the discharge or release of the product from the gland.

Many times their efforts are mutually supplementary but all too frequently the morphologist fails to avail himself of established physiological

observations and similarly morphological data have been overlooked by the physiologist. There are many instances in which the morphologist has first reported the presence of *granular* cells, believing perhaps naïvely, that where there is an accumulation of granules within a cell there is usually secretory activity, and this belief has frequently been confirmed by a later demonstration of the production of secretion. Conversely the physiologists have often demonstrated the presence of a specific secretory product and have in many instances successfully enlisted the aid of the morphologist in locating its source. However, the story does not always have a "happy ending." For example, in the intestine basally granulated enterochromaffin cells and apically granulated cells of Paneth are still without a known function, and similarly the specific morphological source of secretin (the oldest known hormone) is still shrouded in mystery.

I will not attempt to review in any detail the complex and almost endless story of the search by morphologists for the ultimate intracellular source of the individual secretory droplet or granule, but wish to survey some phases of the secretory activity which I hope are nearer the borderline of interest of both the morphologist and physiologist. It is obviously impossible to undertake a comprehensive review and I shall limit myself to the citation of several selected examples which illustrate some of the problems encountered in the study of secretory processes. Before entering upon discussion of these rather special aspects of secretion I should like to satisfy my cytological conscience by referring briefly to the fundamental problem of the mode of origin of the secretory granule.

Secretory granules have a disconcerting habit of suddenly emerging from the submicroscopic to the microscopic level without leaving adequate traces of the point of their origin. Thus it becomes difficult to determine whether they arise directly or indirectly from the nucleus, or from some specifically-formed component of the cytoplasm, or from the optically empty hyaloplasm which is apparently fundamental to the specific organization of all cells.

Almost every formed body within the cell no matter how demonstrated or made microscopically visible, whether in fresh living systems (unstained, or vitally or supravitally stained) or in fixed tissues selectively stained or variously impregnated by compounds of heavy metals, has been designated at some time or other as the source of the secretory product. To my knowledge no one has yet attempted to cite the centriole as being possibly involved in the secretory process but the zone about it, the centrosphere, has been regarded with suspicion by some cytologists.

The succession of theories on the origin of the secretory droplet or granule has paralleled rather closely the advances in cytological technique which have made the several formed-components of the cell distinctly visible. The nucleus (and the nucleolus), the basophilic ergastoplasm, the chondriosomes and finally the Golgi apparatus have successively received intensive study. At the present time the Golgi apparatus is regarded as being the cell component probably most intimately associated with origin of the primordium of the secretory granule or droplet (Kirkman and Severinghaus, 1938). However, Bennett (1940, adrenal cortex, cat) and Payne (1942, anterior pituitary, fowl) find little evidence that the Golgi apparatus participates in the formation of secretion. The primordial vacuole, according to Bowen (1929), arises through the activity of the Golgi substance and undergoes part, at least, of its development in contact with, or embedded in, the Golgi apparatus which thus plays some definite rôle in the process of accumulation and final synthesis of the secretion-product. He does not deny the indirect participation of the nucleus and chondriosomes since all components of the cell possibly contribute in some way.

On the other hand, Parat (1928) places primary emphasis on the vacuole as the center of synthesis and regards the classical Golgi apparatus as a composite artifact made up of vacuoles, special chondriosomes and the diffuse lipoids of the immediate vicinity.

More recently Hirsch (1939) has re-emphasized the importance of individual secretory centers, which arise from a presubstance which in turn is derived from the hyaloplasm or chondriosomes. Each of these secretory centers shows an early differentiation into an internal core, and an external rim which reacts with osmic acid. The secretory product arises from the internal core. According to Hirsch these so-called individual Golgi systems may, in fixed material, be so closely aggregated or agglutinated as to simulate the classical Golgi picture. It is clear that no final disposition of the problem of the rôle of the Golgi apparatus in secretion can be made until cytologists decide whether the Golgi apparatus is a single entity of specific composition, or a complex of several systems closely associated topographically, and perhaps functionally, within the cell.

In the study of secretion the morphologist is largely dependent upon the presence of microscopical evidences of functional activity. Fortunately in many glands the secretory cycle is obvious, with clear histological evidence of elaboration, maturation, accumulation and discharge of the secretory product. In glands of the holo-

crine type, there is but one secretory cycle ending in the dissolution of the cell and the release of the secretion. In other glands (apocrine and merocrine types) the secretory cycle is usually repeated by the individual cells an unknown number of times. In most instances these cycles are distinct and successive but in some cases a new cycle may be initiated before the phase of the preceding cycle has been completed (Brunner's glands, Bensley 1903).

However, well defined secretory cycles are not characteristic of all glands and under normal physiological activity the familiar phases of elaboration, storage and release may be not readily recognized histologically. These phases are either greatly suppressed or absent. Fortunately, in some instances, under specific stimulation, it is possible to so accelerate or exaggerate the secretory process that cyclic phenomena may appear, but in other instances the glands remain refractive to all attempts to evoke a good morphological picture of secretory activity. This is especially true in certain endocrine glands in which secretion may either be continuous with the cells elaborating and discharging their product simultaneously, with no storage; or discontinuous with the level of production so low that no histological evidence of accumulation and discharge occurs. In a few instances, however, as in the thyroid, storage is predominately extracellular or interstitial.

In the first two examples to be discussed the physiological rôle of the glands is well established but the morphological picture of secretion remains relatively obscure.

Usually gland cells show histological evidence of secretory competence, *i.e.*, are specifically differentiated, either before or at the time that the presence of secretory products can be detected. A striking exception to this generalization is presented by the pars intermedia of the pituitary. Chen, Oldham and Geiling (1940) and Rahn and Drager (1941) have demonstrated the presence of melanophore-dispersing hormone in chicks on the fifth day of incubation, before the pituitary gland itself has acquired its definitive form and before any cytological differentiation such as granulation or specific staining reactions have appeared. A similar situation is also present in Amphibia. Kleinholz (1940) has demonstrated the early appearance of the hormone in *Rana pipiens*. Intermedin is detectable in the tail-bud stage with the establishment of only the early buccal ingrowth. This early production of intermedin has been further confirmed by Etkin (1941) in Xenopus and by Drager and Blount (1941) in Amblystoma. It has been demonstrated that secretory competence in this instance is dependent upon contact of the buccal with the infundibular (nervous) component of the pituitary gland and not upon the acquisition of a specific morphological differentiation (Hegre 1942). The recent observations of Gorbman and Evans (1941) on the thyroid of the fetal rat may be contrasted with those on the pars intermedia. By using radio-active iodine these authors determined, by radio-autographs made from histological sections, that iodine was accumulated in the peripheral part of the gland at the 18–19 days of gestation and in the medullary portion at 20–21 days. Follicular differentiation was limited to the periphery of the gland at 18–19 days and at 20–21 days the formation of follicles had begun in the medulla.

Cytological evidences of functional activity of the parathyroid gland are also relatively meager. Rosof (1934) found in the parathyroid of the rat, following impregnation with osmic acid, that the chief cells were of two types, light and dark. The dark cells, filled with an osmiophilic substance, were interpreted by Rosof as a phase of functional activity of the light cells. Normally from 15 to 25 *per cent* of the cells are dark. More recently De Robertis (1940) has utilized a large single dose of parathyroid extract (1000 u. administered over a period of 5 hours) to depress parathyroid activity. Clear cut histological changes were observed 6 hours after the last injection and there were increasing evidences of hypofunction up to the fifth day when no dark cells were seen and the cellular picture was homogenous, *i.e.*, all cells seemed to be in a resting state. After the fifth day there were increasing evidences of resumption of function and at the fifteenth day the cellular pattern was characteristic of the normal gland. In the hypofunctional state the cells are small, with uniformly simplified Golgi and mitochondrial patterns. With recovery the homogeneity of the cellular picture disappeared and the cells were at various levels of activity—characterized by increase in size, with fragmentation and vacuolation of the Golgi apparatus and vesiculation of the chondriosomes. Dark cells reappear. The vacuoles associated with the Golgi material and mitochondria seem to be the only evidences of secretory activity. No specifically stained globules or granules could be demonstrated.

De Robertis (1941) also produced an exaggerated picture of parathyroid activity by placing rats on rachitogenic diets of either low phosphorus or low calcium content. Evidences of hyperfunction were greater in low calcium rickets and in some cases there were 50 *per cent* of dark cells instead of the normal 15 to 20 *per cent*. Even in this extreme activation no granules that could be interpreted as secretory were found although the usual vacuoles in association with fragmented Golgi material and mitochondria were present.

Recently, however, Grafflin (1940, 1942) has found basophilic granules in the parathyroid cells of the deer which he regards as probable evidence of secretory activity.

In the next example selected, the adrenal cortex, the morphological expression of secretory activity is much more obvious and interest centers about the life history of the secreting cells. There is accumulated evidence that in this component of the adrenal gland the secretory cycle is coincident with the life cycle of the cortical cells; i.e., the several cell types seen in the several zones of the cortex represent different stages in the life history of the cells. These cells arise by mitotic division of elongated capsular or subcapsular cells and migrate centripetally to the cortico-medullary junction near which they undergo degeneration, but there may also be some increase in their number by mitosis in the glomerular and outer fascicular zones (Hoerr 1931).

Bennett (1940), by appropriate histochemical tests, has determined the distribution of the biologically active sterones within the adrenal cortex of the cat and on the basis of these studies has recognized four distinct zones, "presecretory," "secretory," "post-secretory" and "senescent," respectively. These zones based on a functional classification do not coincide with classical zones of Arnold (glomerulosa fasciculata and reticularis) based on the morphological patterns assumed by the cortical cells, but seem likely to supersede the morphological terminology. Bennett gave careful attention to the problem of the mechanics of the life cycle of the cortical cells, accepting the interpretation that the cells are continuously migrating from their capsular point of origin toward the medulla. He recognized that by making certain corrections such as for changes in the volume of the cells it should be possible to make certain deductions concerning the relative velocities with which cells might pass from one zone to another and hence to determine the relative length of time taken by a cell to migrate through a certain zone—based upon the relative thicknesses of specific zones. Accordingly hypertrophy and atrophy of the cortex must be interpreted dynamically and primarily in relation to the actual relative thicknesses of the several zones before any conclusion can be drawn regarding possible increase or decrease of hormone production.

More direct information on the problem of rate of migration of cortical cells from the capsule toward the medulla, has been obtained by Salmon and Zwemer (1941) for the adrenal cortex of the rat. Following injections of trypan blue, small blue droplets accumulate in the fibroblast-like cells of the capsule. Thus by using appropriately spaced injections these investigations were able to follow the rate of migration of marked cells toward the medulla. They conclude: "The schedule of the life cycle of an adrenal cell in the series of rats used would be about as follows: from the capsule to the glomerular zone—average 6 days; from capsule to beginning of fascicular region—average 12.2 days; from capsule to reticular layer 20 to 30 days (p. 425).

Accordingly, in the interpretation of experiments involving the adrenal cortex, it must be recognized that individual adrenal cells pass through only one cycle and that the cells of the succeeding cycle must move into the secretory zone before a new activity can occur. The response therefore will probably be relatively slower than that of a gland in which the fully competent secretory cells may pass through repeated cycles of secretion.

In the fourth example I wish to focus attention on the difficulty of ascertaining the specific source of a secretory product in a gland in which the number of products exceeds the number of different cell types composing the gland. At the present time the number of biologically active principles obtained from the anterior pituitary gland greatly exceeds the number of cell types that can be differentiated by any of the many selective stains in common use. Only three chief categories of cells, designated by various letters and numbers but usually referred to as acidophiles, basophiles and chromophobes, are generally recognized. The two chromophilic types are the only cells regarded as engaged in secretory activity; the chromophobes are thought to be inactive phases of these types. The chromophilic cells may exhibit great variations in cell size, size of specific granules, degree of granulation and degranulation, in mitochondrial content, and in the size and patterns of the Golgi apparatus. However, they do not show staining reactions which permit them to be separated tinctorially into as many sub-groups as there are numbers of active principles. This situation is not peculiar to the anterior pituitary. A similar condition is found in the exocrine pancreas which produces three and perhaps four specific enzymes but the zymogen granules of all cells are alike both in size and tinctorial reactions and these ferments are probably as different chemically as are the several principles of the anterior pituitary. Furthermore other zymogen granules, such as the propepsin and proptyalin types are also similar in size and staining capacity.

Recently following formalin fixation, or fixation in corrosive sublimate-formol, followed by Heidenhain's "Azan" modification of Mallory's triple stain, Dawson and Friedgood (1938) have successfully differentiated two distinct tinctorial types of acidophiles, a special type reacting spe-

cifically with azocarmine and the ordinary type, staining with orange G. This method is very similar to the one utilized by Bloom (1931) to demonstrate alpha, beta and delta cells in the islets of Langerhans. These two tinctorial types of acidophiles have been recognized chiefly in the anterior pituitary of the female rabbit, cat, ferret and monkey and the presence and numbers of the so-called carmine cell have been correlated with definite stages of reproductive activity. Our observations are most extensive for the rabbit and cat (Friedgood and Dawson, 1938, 1940).

In the rabbit, the pituitary is stimulated by coitus and this results in the induction of ovulation. The carmine cells are present in moderate numbers during estrus and are significantly increased in number within one-half hour after mating. They usually appear aggregated into compact, irregularly localized areas. Preceding ovulation a decided degranulation occurs.

In the cat, carmine cells are absent in anestrus and they first appear in limited numbers and in constant areas in the proestrus animal and then increase in number and extent in estrus. After mating there is again a considerable increase in numbers and distribution, reaching a maximum within 5 to 6 hours. A significant preovulatory degranulation also occurs. These carmine cells may reappear in relatively large numbers during the period in which the ovum is passing down the tube to become implanted. After implantation they are relatively sparse until the end of the third week of pregnancy when they appear in gradually increasing numbers until they dominate the picture during the last week of gestation. They are not materially changed during parturition and exhibit increasing degranulation and decrease in numbers during active lactation. When kittens are not permitted to nurse at all these cells show regression rather than degranulation. This phenomenon is readily recognized as early as 48 hours postpartum and is quite marked by the sixth day.

The precise functional significance of these cells exhibiting such an affinity for azocarmine is not known. Correlation of physiological and cytological data suggest that the post-coital or preovulation response is related to the release of the luteinizing hormone. The significant increase in their number during late pregnancy and their persistence in the lactation period indicate that they may be associated in some way with other aspects of reproductive activity. The morphological basis of the carmine reaction is undetermined. No transitional stages between ordinary acidophiles, reacting with orange G, and the carmine cells have been observed, yet the rapid postcopulatory response suggests that one tinctorial type must transform rapidly into the other. This is especially true in the rabbit. In the cat on the other hand, the number of chromophobes in the anestrus period is very great and their localization in the pituitary coincides with the sites of the early appearance of the carmine cells. It is possible that these cells may arise both by the specific granulation of chromophobes and by the transformation from one tinctorial type to the other. The disappearance of carmine cells, however, is accomplished usually by degranulation although in a non-lactating, post-partum cat there are obvious regressive changes. This limited tinctorial distinction of two acidophile phases is encouraging and lends hope that with newer methods other histological evidences of differential functional activity may be found both in the acidophilic and basophilic cells.

I present the last three examples with some misgivings. They are not long-standing members of the community of glands and it may be that they do not have all the qualifications for admission to this group. However, in any present day discussion of secretion some passing reference should be made to the so-called neurosecretory cells—cells which in addition to their nervous character give histological evidence of gland-like activity. A rather voluminous literature has grown up on this subject since the early observations of Dahlgren (1914) and Speidel (1919, 1922) on the occurrence of such cells in the spinal cord of certain elasmobranches. Excellent reviews by Scharrer and Scharrer (1938, 1940) have appeared recently. These cells have a wide distribution in nervous tissue and frequently are grouped in definite loci in the central nervous system of many invertebrates (annelids, molluscs and arthropods) and in all classes of vertebrates with the possible exception of the birds. In several instances good morphological evidence of cyclic activity has been obtained and there is much to suggest that the materials elaborated within these modified nerve cells are released into the circulation.

In insects these neuroglandular elements apparently exert an endocrine control over such proccesses as molting and pupation, and in Limulus (Scharrer 1941; Brown and Cunningham 1941) they are regarded as the source of a chromatophorotropic substance, capable of influencing color changes in crustacea. In fish and amphibia the neurosecretory cells are most consistently found in the nucleus preopticus, while in reptiles and mammals they are readily demonstrated in the nuclei supraopticus and paraventricularis. In the vertebrates, however, there is as yet no information either on the nature of the substances produced by these glandular regions or on their physiological significance. It is to be hoped that more investigators will be attracted to this problem of neuronal secretion.

In addition to this concept of neurosecretion it appears that we may have to consider that of myosecretion. I refer to the specifically modified cells in the wall of the preglomerular segment of the afferent arteriole of the mammalian kidney. This part of the afferent arteriole is characterized by a change in the form of the endothelial cells, by the loss of the elastic membrane and the modification of the media. Here the normal smooth muscle fibers are in part replaced or overlaid by afibrillar or myoepitheloid cells which in renal ischemia exhibit gland-like activity. This portion of the arteriole also lies in close anatomical association with a locally modified portion of the wall of the distal convoluted tubule, in the region of transition from ascending limb to distal tubule. In this special region of the tubule the epithelial cells are usually taller and narrower with closely spaced nuclei, forming a plaque-like structure, the macula densa. The macula densa and the epithelial-like cushion on the pole of the afferent arteriole are the chief components of the so-called juxta-glomerular apparatus which with the associated nerve supply was believed to constitute a local mechanism, neuro-myo-arterial apparatus, for the regulation of the glomerular circulation, under the influence of such factors as the composition and volume of the tubular urine.

However, it is in renal ischemia, either pathological or experimental, that the myoid cells of the preglomerular segment of the afferent arteriole exhibit their most complete transformation into granular, gland-like cells. Accordingly, the present, tentative, interpretation is that these glandular, preglomerular segments constitute a diffuse endocrine system liberating a pressor substance into the blood stream and producing thereby a generalized circulatory effect rather than controlling separately and locally the circulation of individual glomeruli.

The granular, and implied glandular, nature of the cells on the arteriolar segment was first recognized by Rüyter (1925) in the kidneys of rats and mice.[1] Later Oberling (1927) found a similarly modified segment on the preglomerular segment of the human kidney. Rüyter's observations on the mouse kidney were later verified by Okkels (1929) who also recognized a comparable structure at the vascular pole of the glomerulus of the frog. In 1932 Goormaghtigh was able to confirm Oberling's studies on the human kidney and also found a similar condition in the cat. In the following year Zimmermann made an extensive survey of a variety of mammals and concluded that the modified preglomerular arteriolar segment was a consistent feature of the mammalian kidney. He was also the first to recognize the frequent, anatomical, association of the modified arteriolar wall and the macula densa of the uriniferous tubule.

We appear to be indebted to Goormaghtigh and his associates for the development of the concept of the endocrine nature of the myoepitheloid cells (Goormaghtigh 1937a, 1937b; Goormaghtigh and Handovsky 1938, Goormaghtigh 1939a, 1939b; Goormaghtigh and Grimson 1939, Goormaghtigh 1940a, 1940b). They demonstrated that in renal ischemia there is hyperplasia and hypertrophy of the afibullar cells which rapidly becomes heavily granulated. These cells appear to be derived from typical smooth cells of the media which lose their fibrillae and spindle-form, and acquire granules which are first acidophilic, then basophilic and vacuolated. These cytological observations have been further confirmed by Dunihue and Candon (1940) in experiments on the rabbit. Since renal ischemia stimulated almost exclusively the cells of the media of the arterioles Goormaghtigh believes the source of the hypertensive substance must be sought in these cells. However, Edwards (1942) concludes that there is no unequivocal evidence of continuous or periodic secretory activity in this region and is inclined to regard the macula densa of the tubule as a more likely site of a pressor substance of renal origin. His conclusions are based on kidneys of normal and hypertensive persons and normal and renally hypertensive dogs and rabbits.

Our knowledge of the juxta-glomerular apparatus is confined chiefly to the mammalian kidney, although Okkels (1927) refers to a modified preglomerular segment in the afferent arteriole of the frog and more recently Edwards (1940) has described a typical plaque or macula densa in the kidney tubule of this animal. Edwards also studied animals typical of the other classes of vertebrates and found that both the arteriolar pad and the epithelial plaque of the tubule were also present in the avian kidney but both were totally lacking in fishes[2] and reptiles. He also pointed out that in both mammals and birds there are many exceptions and variations in the associa-

[1] I am indebted to Mrs. Ruth Castle Griffin for many of the references cited here.

[2] Friedman and Kaplan (1942) have reported the presence of renin in the glomerular kidneys of the carp and catfish and its absence from the aglomerular kidney of the midshipman fish. These observations suggest that the production of renin is associated with the presence of glomeruli rather than with tubular activity. However, only limited numbers of juxta glomerular or periarteriolar cells were observed in the glomerular kidneys and none of these cells gave histological evidence of secretory activity.

tion of the plaque and pad. In mammals the plaque may be absent twice as often as the pad, while in birds the plaques are present twenty-five times as often as the pad. The variability in the association of the plaque and pad must be of some significance and suggests that they may not be as closely associated functionally as was thought by the earlier investigators. It is clearly indicated that this aspect of kidney morphology deserves more intensive study if we are to determine the source of the product which makes its appearance in the blood stream following radical interference with the normal hemodynamics of this organ.

Finally I should like to direct your attention to a still more diffuse system of cells whose distribution is not limited to any one organ but which are found in almost all loose connective tissue and frequently localized in close association with the smaller divisions of the vascular system—the mast cells of Ehrlich. Michels (1938) in a comprehensive review enumerates twenty-five hypotheses regarding the possible functions of the mast cells and concludes "that obviously we are far from knowing their biological significance" (p. 310) and states further, "As yet no one has shown that mast cells exhibit rhythmic quantitative changes in their granule content, a requisite before the secretion process can be admitted" (p. 314).

Recently, however, attention has been refocussed on these cells—as the probable source of heparin. This has largely been the result of a series of studies by a group of Scandinavian investigators (Holmgren and Wilander 1937; Jorpes, Holmgren and Wilander 1937, Jorpes 1939). Much of the difficulty in demonstrating mast cells in routine paraffin sections was overcome by a new method of fixation (10 *per cent* solution of basic lead acetate) followed by staining in an alcoholic solution of toluidin blue which stains the granules strongly and metachromatically. It was also found that purified heparin responded similarly to toluidin blue and that the presence of heparin could thus be directly demonstrated in the walls of many large blood vessels. Furthermore a rather good correlation was found between the density of the population of mast cells of various tissues and organs and their yield of heparin. The evidence is strongly suggestive that the granules of the mast cells of Ehrlich are associated with the elaboration of heparin.

Nevertheless many factors, such as absence of any demonstrable secretory cycle, great variations in distribution and number in the various tissues and organs and variations in the absolute numbers present in certain species (sparse in the rabbit), make it necessary to make certain reservations before fully accepting mast cells as the specific source of heparin. The need of all animals for this compound would appear to be relatively constant but morphological data show that the mast cell population in vertebrates fluctuates greatly from both class to class and from species to species.

I had hoped to find time to discuss still another phase of the problem of secretion, *i.e.*, the evidences of the length of life of the individual secretory units and the provision made for the maintenance of a normal secreting population, but I must refrain from introducing an additional topic since this discussion already includes too many diverse aspects of the problem.

In this review instances of secretory activity in which the findings of the morphologists and physiologists are in good agreement have been purposely omitted. Consideration has been given to examples in which information on either morphology or physiology is deficient or in which it has not been possible to correlate adequately the findings of the two groups of investigators. No single theme runs through this paper and no general conclusion can be made at its close, unless it is that there is still need for close collaboration between histologists and physiologists if biological problems such as secretion are to receive final solution.

REFERENCES

Bennett, H. S. Am. J. Anat. **67:** 151, 1940. Bensley, R. R. Univ. of Chicago Decennial Publ., S. 1, **10:** 277, 1903. Bloom, W. Anat. Rec. **49:** 363, 1931. Bowen, R. H. Quart. Rev. Biol. **4:** 299, 484, 1929. Brown, F. A., Jr. and O. Cunningham. Biol. Bull. **81:** 80, 1941. Chen, G., F. K. Oldham and E. M. K. Geiling. Proc. Soc. Exper. Biol. and Med. **45:** 810, 1940. Dahlgren, U. Science **40:** 862, 1914. Dawson, A. B. and H. B. Friedgood. Stain Techn. **13:** 17, 1938. De Robertis, E. Anat. Rec. **78:** 473, 1940; **79:** 417, 1941. Drager, G. A. and R. F. Blount. Anat. Rec. **81:** Suppl.: 92, 1941. Dunihue, F. W. and B. H. Candon. Arch. Path. **29:** 777, 1940. Edwards, J. G. Anat. Rec. **76:** 381, 1940; **82:** Suppl.: 462, 1942. Etkin, W. Proc. Soc. Exper. Biol. and Med. **47:** 425, 1941. Friedgood, H. B. and A. B. Dawson. Endocrinology **22:** 674, 1938; **26:** 1022, 1940. Goormaghtigh, N. Arch. de Biol. **43:** 575, 1932; J. Physiol. **90:** 63, 1937; Proc. Soc. Exper. Biol. and Med. **42:** 688, 1939; Brux. Méd. **19:** 1541, 1939; Am. J. Path. **16:** 409, 1940; Arch. de Biol. **51:** 293, 1940. Goormaghtigh, N. and K. S. Grimson. Proc. Soc. Exper. Biol. and Med. **42:** 227, 1939. Goormaghtigh, N. and H. Handovsky. Arch. Path. **26:** 1144, 1938. Gorbman, A. and H. M. Evans. Anat. Rec. **81:** Suppl.: 95, 1941. Grafflin, A. Endocrinology **26:** 857,

1940; Anat. Rec. **82:** Suppl.: 466, 1942. HEGRE, E. S. Anat. Rec. **82:** Suppl.: 420, 1942. HIRSCH, G. C. Form- und Stoffwechsel der Golgi-Körper. Berlin, 1939. HOERR, N. Am. J. Anat. **48:** 139, 1931. HOLMGREN, H. AND O. WILANDER. Ztschr. f. mikr.-anat. Forsch. **42:** 242, 1937. KLEINHOLZ, L. H. Biol. Bull. **79:** 432, 1940. JORPES, E. Heparin, its chemistry, physiology and applications in medicine. London, 1939. JORPES, E., H. HOLMGREN AND O. WILANDER. Ztschr. f. mikr.-anat. Forsch. **42:** 279, 1937. KIRKMAN, H. AND A. E. SEVERINGHAUS. Anat. Rec. **70:** 413, 557, 1938; **71:** 79, 1938. MICHELS, N. A. Handbook of hematology. H. Downey, ed., **1:** 235, 1938. OBERLING, C. R. C. R. Acad. Sci. **184:** 1200, 1927. OKKELS, H. Bull. d. Hist. appl. **6:** 113, 1929. PARAT, M. Arch. d. Anat. Micr. **24:** 73, 1928. PAYNE, F. Biol. Bull. **82:** 79, 1942. RAHN, H. AND G. A. DRAGER. Endocrinology **29:** 725, 1941. ROSOF, J. J. Exper. Zoöl. **68:** 121, 1934. RÜYTER, J. H. C. Ztschr. f. Zellf. u. mikr. Anat. **2:** 242, 1925. SALMON, T. N. AND R. L. ZWEMER. Anat. Rec. **80:** 421, 1941. SCHARRER, B. Biol. Bull. **81:** 96, 1941. SCHARRER, E. AND B. SCHARRER. Biol. Rev. **12:** 185, 1937; Res. Publ. Ass. nerv. ment. Dis. **20:** 170, 1940. SPEIDEL, C. C. Publ. Carneg. Inst. **13:** 1, 1919; J. Comp. Neurol. **34:** 303, 1922. ZIMMERMANN, K. W. Ztschr. f. mikr.-anat. Forsch. **32:** 176, 1933.

PHYSIOCHEMICAL ASPECTS OF THE SECRETORY PROCESS

RUDOLF HÖBER

Department of Physiology, University of Pennsylvania

I am going to define the secretory process as a process changing the spatial distribution of dissolved substances in passing a layer of epithelial cells, which separate two aqueous solutions, and I am going to analyze the factors controlling the distribution. One may hesitate to admit such an utterly simple definition. As a matter of fact, it frees me from considering the more chemical problem of the new formation of specific components in the secretory process (like enzymes, lactose, bile salts) and leaves me the task of turning the light on the physico-chemical forces involved in the most variable distribution of the pre-existent substances, by studying glands and gland-like plant tissues.

On the basis of our present knowledge we may say that the difficulties arising in such an analysis are found on two fairly distant levels of complexity. On the lower level we meet the *passive penetration* of the secretory cell layer, on the upper level we are faced with what may be called the *active transfer*.

Passive penetration in the case of nonelectrolytes means a spontaneous approach to an equilibrium; this means equality of concentration on both sides of the membrane, provided that no chemical reactions interfere. The main properties of the solutes involved in the rate of approach to an equilibrium are the molecular volume and the lipoid solubility. For instance, the cells of the isolated submaxillary gland, due to their porous structure, do not allow hexose to pass from the perfusing fluid into the secretion, but permit the triose dioxyaceton because of the differences in the molecular volume. Butyramide having rather the same molecular size as malonamide, has a much higher penetrating power than the latter, owing to its lipoidsolubility (1).

Much more significant, but much more difficult to analyze is the *active transfer*. In general, it results in an unequal distribution because of a shift of certain solutes up-hill against a concentration gradient. This needs the expenditure of energy; it means, in general, a supply of fuel. However, a movement against a concentration gradient is by no means always due to an energy supply. Particularly, as far as ions are concerned, the Donnan effect should be taken into consideration and it should be tested as to whether the characteristically different Donnan distribution of cations and anions appears or not.

An event, happening probably more frequently than observed, is that passive penetration and active transfer are running together. For instance, while perfusing an isolated frog liver with Ringer solution with the addition of lactose and an adequate dyestuff, the lactose, by passive penetration, reappears in the secretion at the perfusion concentration, the dye, by active transfer, at a concentration several hundred times higher (2).

In the following survey chiefly the active transfer will be analyzed, and I propose to discuss mainly three points: 1, the substances undergoing the secretory transfer; 2, the linkage between the active transfer and the nature of the supplied energy; 3, some concepts regarding the transferring devices, the secretory machine,—so to speak.

The substances involved in active transfer. Selective effects are nowhere a more familiar phenom-

enon than in pharmacology, where in numerous cases the site of action displays some kind of specific affinity between drugs and cells. Selective transfer is most evident to physiologists in the action of the kidney as the principal osmotic machine, where, due to various cellular devices located along the nephrons, certain plasma components are shifted into the tubular fluid and others are returned to the plasma. In order to correlate the specific transporting capacity of the cells to the chemical and physico-chemical properties of substances, which are being selected for transport in one or the other direction and to a greater or a smaller extent, it has been found useful for more than 60 years to follow the path of dyestuffs, even though these substances are foreign to the body. The selective behavior of the frog kidney towards dyes is roughly demonstrated in table 1. All the dyestuffs listed are sulfonic acid dyestuffs, which, contrary to the basic dyestuffs, in general fail to enter and to pass living cells, but can be accepted by certain cells, e.g., the epithelia of the proximal convoluted tubules, provided they own an adequate molecular structure. The numerical values in this table indicate accumulation ratios. It appears that the concentration of some of the dyestuffs has been raised to a level high above that of the perfusion fluid, some appear slightly concentrated and some fail to appear at all.

TABLE 1

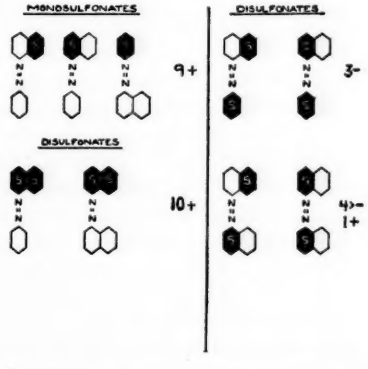
Selective transfer of dyestuffs by the frog kidney

	CONCENTRATION RATIO		CONCENTRATION RATIO		CONCENTRATION RATIO
Azofuchsine B	15, 5, 15, 15	Ruby G	2, 1, 2, 2	Cyanol Extra	0, 0, 0, 0
Ponceau R	20, 15, 12, 60	Fast Red E	5, 3, 1, 1	Azofuchsine IV	0, 0, 0
Azofuchsine I	30, 50, 20, 40	Erythrin 2R	1, 1, 5, 1	Azofuchsine G	0, 0, 0
Palatine Red A	25, 15			Wool Green S	0, 0, 0
Scarlet 3BX	15, 10, 50, 15			Eriocyanin A	0, 0, 0, 0
Palatine Scarlet	20, 10, 3, 24				

What is the reason for this variety of behavior? The molecular weights of these dyestuffs do not differ much. They are well diffusible and they are lipoidinsoluble. There have been investigated dyestuffs belonging to various classes, among them azo- and disazo-dyestuffs (3). Let us consider the azodyestuffs. As table 2 shows, their molecules are characterized by a bilateral and probably elongated configuration. Both molecular halves have a ring structure and are linked together by an azo-group. It appears that special importance has to be assigned to the sulfonate group, to their number as well as to their location in the structure. On the left side of the table there are listed the diagrams of 19 different mono- and disulfonates with the sulfonate groups located in one molecular half. All of them undergo secretory concentration as marked by a + sign. On the right side there are put together the diagrams of 8 disulfonates, the sulfonates distributed in such a way that either half-molecule bears one sulfonate radical. These dyestuffs either fail to be secreted or they pass only to a small extent, except one dyestuff, a Crocein Scarlet,—for an unknown reason. This behavior has suggested an interpretation following the concepts developed by Langmuir and Harkins: the dyestuffs pictured on the left side of the table have the character of a dipole, the sulfonated molecular half being polar and hydrophilic, the non-sulfonated one being nonpolar and hydrophobic-organophilic. Therefore, it can be assumed that the selective accumulation of the nonpolar-polar dyes is due to a preliminary orientation and attachment of the molecules to the acting organic structure, probably to the cell surface, by the organophilic half, while—by a suitable partial rotation of the elongated molecules,—the hydrophilic half could be anchored in the aqueous surroundings. This loose attachment by van der Waals forces is assumed to precede the active transfer. A convincing demonstration of the contrasting behavior of the hydrophilic-hydrophobic and the hydrophilic-hydrophilic dyes is the following: Richards and Barnwell (4) have shown that an excised frog kid-

TABLE 2

Sulfonic acid azodyestuffs



ney immersed in oxygenated Ringer continues to act, when exposed to phenol red. Following this procedure, after having placed a kidney into the violet mixture of the blue nonpolar-polar dye Cyanol and the red polar-polar Azofuchsin G for half an hour, you find the tubular lumina filled with blue color as the result of a physiological demixture.

It must be admitted that, so far, in more than one respect there are not experimental data enough to give a solid fundament to the proposed hypothesis. In order to strengthen and, if possible, to generalize the idea of a nonpolar-polar structure being an essential factor in the secretory act, particularly in order to approach somewhat more the problem of physiological transfer, the study has been extended to organic compounds more simple than the dyestuffs, namely, to the naphthalene- and benzenesulfonates, which, on one hand, resemble the azodyes as being their molecular halves and which, on the other hand, are relatives to the large group of sulfanilamides. These benzene- and naphthalenesulfonates were chosen for a technical reason: after having supplied the frog kidney with one naphthalenesulfonate, its concentration in the resulting secretion can be determined by adding to the secretion another sulfonate known to yield in a diazotation a colored product, an azodyestuff, so that a colorimetric determination of the organic sulfonate in question is possible.

The simple result of this study (5, 6) is shown in tables 3 and 4. It appears that the naphthalene-*mono-sulfonates*, in passing the wall of the proximal tubules, become concentrated, whereas the naphthalene-*di-sulfonates* do not pass. This is unexpected insofar as it appears to be irrelevant or, more correct, not markedly relevant, where the sulfonate groups are located in the ring system, with regard to the NH_2 and OH radicals present.

Is it permissible to argue that also in these secretory processes a nonpolar-polar affinity is involved? In other words: conceiving the naphthalene nuclei, including the NH_2 and the OH groups, as being more or less organophilic and the sulfonates as being hydrophilic, does the pull of two sulfonates towards the water outweigh the adhesive forces of the organophilic part, while the pull of one sulfonate does not? This may sound somewhat hazardous. But, this interpretation can be supported by various observations. *First*, by the fact that, as shown in table 4, substituting the naphthalene with the benzene ring prevents the monosulfonates from being secreted, and there are several reasons to believe that the attachment of the benzene ring to the cell surface is weaker than that of the naphthalene. One of these reasons is the following: when the balance between the opposing organophilic and hydrophilic forces is shifted towards the organophilic part by attaching to it an alkyl radical,—let us say—a butyl group, the resulting alkyl-aryl-sulfonates become dispersing agents, the dispersion, for instance, being directed towards the surface membrane of muscle fibers, and and the strength of this dispersing or loosening effect depending upon the length of the carbon-atom chain as well as upon the ring system present. It appears that, the alkyl carbon-atom chain being the same, e.g., a butyl, the naphtha-

TABLE 3

Secretability of dyestuff intermediates I

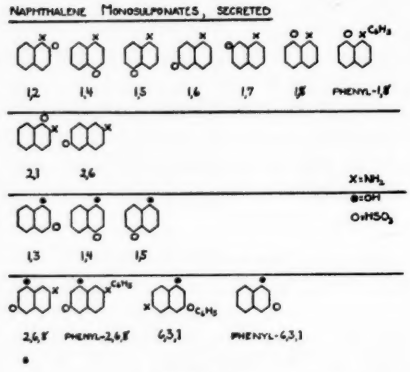
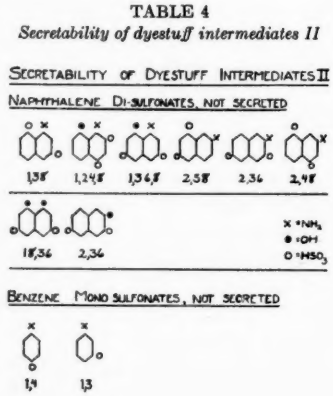


TABLE 4

Secretability of dyestuff intermediates II



lene compounds display a greater, but reversible injury potential and correspondingly a stronger inhibitory effect on muscle contraction that the benzene compounds do. *Second:* it is enough to attach to a benzene-sulfonate a second separate benzene ring to get the same effect as with a naphthalene sulfonate. This is shown in table 5.

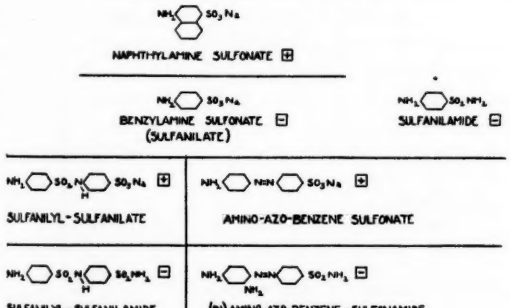
A plus sign marks a secretory concentration, a minus sign the contrary. Starting with an amino-naphthalene-sulfonate, which is concentrated, the corresponding amino-benzene-sulfonate is not. However, after having increased the organophilic affinity by the attachment of a second benzene

ring, the resulting compounds, sulfanilyl-sulfanilate and amino-azo-benzene-sulfonate, are concentrated. *Third:* either of these compounds has an organophilic-hydrophilic structure. This can be abolished more or less, e.g. by substituting the sulfonamide group for the sulfonate group, as it appears in disulfanilamide and in prontosil. The effect is a deprivation of the kidney from showing its transporting capacity.

So much about the nonpolar-polar molecular configuration as correlated to the kidney activity. I wish to turn now briefly to another gland, the liver, where the useful tool of the dyestuffs gives clear evidence that in this gland entirely different laws are governing the action. The isolated Ringer perfused frog liver displays the amazing capacity to concentrate dyestuffs to more than a thousand times the perfusing concentration, but, seemingly, irrespective of their molecular configuration. So far, there were mentioned only azo dyestuffs. It is irrelevant to the liver,

TABLE 5

Nonpolar-basic—polar-acidic compounds



whether these are nonpolar-polar or polar-polar. So far, there was not mentioned the discriminate behavior of the sulfonic acid triphenylmethane dyestuffs towards the kidney. Some are excreted, others are not. But the liver does not make any distinction among them. It concentrates them, unless, for some unknown reason, they are poisonous or they are retained in the liver as due to a high solubility in the fatty substances, or they fail to pass because they are highly colloidal. There is reason to believe that the great adsorbability of the dyestuffs, so frequently observed, is an important factor in the transfer across the hepatic epithelia, because, on one hand, substances like sugars or inorganic ions, typically low in adsorption affinity, pass across the surviving perfused liver as if across a filter (2), and, because on the other hand, polysulfonate dyes—and this means highly hydrophilic substances,—are excluded from the secretory accumulation (8).

But, beside adsorption as an integrating factor in the process of dyestuff secretion, actual chemical forces are known to be involved in the transfer, and in concluding this first section about physico-chemical properties qualifying for a selective transfer, I may say that numerous remarkable observations about the selective transfer of inorganic ions and of some nonelectrolytes, e.g., urea, as described in the work of Hoagland (9), Steward (10), Krogh (11), Visscher (12), Davenport (13) and others, are omitted in this survey for no other reason than that so far little evidence has been afforded about physico-chemical factors being participant in this selection.

The energy available in the active transfer. In the following paragraph we will deal with the energy available for the active transfer. In the studies of F. C. Steward and D. R. Hoagland this correlation has been demonstrated in a most conspicuous and convincing manner with plant tissues. The main subject of Steward (10) were potato discs of one mm and less in thickness, placed in water and well aerated for several days. Under these conditions the dormant starch-filled

TABLE 6

Carrot discs in KBr solution. Time 69.5 hours

EXTERNAL CONCENTRATION	FINAL INTERNAL CONCENTRATION	ACCUMULATION RATIO
0.20×10^{-3}	2.49×10^{-3}	12.45
2.00×10^{-3}	14.40×10^{-3}	7.22
20.00×10^{-3}	68.00×10^{-3}	3.41

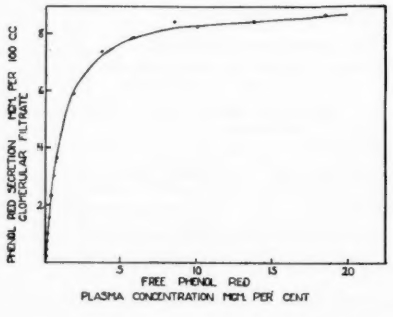
storage cells become more and more depleted from starch, their respiration increases, protoplasm streaming becomes visible and simultaneously the cells begin to transfer cations and anions into the cell sap from very dilute salt solutions, e.g., K and Cl. They pass across the thin protoplasmic wall and in doing so they accumulate in the sap in a concentration many times higher than being present in the surroundings. Hoagland (9) utilized as his favored subject, in several respects superior to the potato discs, excised roots of barley seedlings grown in water. The following table shows the uptake of potassium bromide by carrot discs in relation to the external concentration after 66 hours. It appears that the lower the outside concentration is, the higher is the accumulation ratio. The highest value in the table is 12.5. But, even a shift of inorganic ions up-hill to about 1000 times can be observed, provided that the outside concentration is very low, and since conductivity measurements have proved the ions accumulated in the cell sap to be there in free solution, work must have been done, some kind of energy must have been expended, free

energy has been gained. These events appear to be very much like those observed in animal tissues, for instance, the striking up-hill shift of sodium chloride in the distal tubules of the Ringer perfused frog kidney or the accumulation of dyestuffs by the corresponding preparation of the frog liver. Also the correlation between the accumulation ratio and the concentration available to the cells from outside (table 6) is well known in animal physiology. We will come to this point later.

The next question regarding the nature of energy expenditure has been studied in many ways. In the experiments of Steward and of Hoagland, obviously, the answer is that the energy is derived mainly from aerobic carbohydrate metabolism, although not exclusively. Carbohydrates likewise are the main source of energy for the active transfer in animal tissues. An excellent subject for study of this correlation is the mesonephros of the chick embryo, as utilized by Robert Chambers (14). Explanted pieces of the proximal tubules of this embryonic tissue grow out to form epithelial cysts, which display their secretory activity by transporting dyestuff from outside and accumulating it inside the lumen. This action can be diminished or even stopped by lack of oxygen or by cyanid, and it is particularly indicative of the utilization of carbohydrates that the action also can be stopped by iodoacetate and, in presence of iodoacetate, can be reactivated by adding proper amounts of lactate or of pyruvate (15). This has been found to be true also with the isolated perfused frog liver (16) and cat's salivary gland (17). Low temperature is another means to stop the active transfer, not only to slow it down. Collander has observed that, in higher plants, special cells in the neighborhood of the vascular bundles have the capacity to store in their sap sulfonic acid dyestuffs, as liver and kidney do in their secretions. This action can be entirely suppressed not only by anaesthetics, but also by low temperature (18). An interesting series of experiments has been carried out by Hoagland and Davis (19, 20). The fresh water alga Nitella is significant in that its giant cells are large enough to draw, for chemical analyses, sufficient amounts of sap from their vacuole. After being kept for a while in the dark in very dilute KCl solutions, the cells at daylight transfer and accumulate Cl, eventually to completeness. At low temperature this Cl leaks out and can be recaptured at normal temperature. The favorable influence of light relies, partially at least, upon the photosynthetic reactions and the liberation of oxygen. Also, the kidney epithelia stop doing their work at low temperature.

Some concepts regarding the transferring devices. I have to turn now to the last chapter dealing with some concepts regarding physico-chemical aspects of the transferring machine. This is in reality not more than a glimpse, since so far our knowledge is very poor. According to the aforementioned experiments with certain dyestuffs and some other compounds, a nonpolar-polar molecular configuration seems to be an essential factor in initiating the transfer. This probably means an attachment to the cell surface by adhesive affinities, forces not too strong, since the opposing affinities, organophilic on one side, hydrophilic on the other, as exhibited by a nonpolar-polar molecule, would disrupt the normal organization of the cell surface, as the nonpolar-polar dispersing, wetting and detergent agents really do. The adsorption, then, as a first step in the complex process, could perhaps be thought of as being succeeded by a shift of the compounds in question, as a load attached to a movable carrier. Such concept has repeatedly been put forward and has received support from experiments cor-

TABLE 7



relating the concentration of substances available for the active transfer on one side of the cell layer and the absolute amounts delivered on the other side. These experiments, as performed by Marshall and Crane (21), Scheminsky (22), Steward (10), Verzar (23), Donhoffer (24) and especially thoroughly by Shannon (25), gave evidence that, gradually increasing the concentration of the supplied solution, the rate of the active shift reaches a constant maximal value, depicted graphically as in the slide representing an experiment of Shannon about the excretion of phenol red by a dog's kidney (table 7). On the abscissa there are plotted the mgm. per cent of free dyestuff provided for the kidney by the blood, on the ordinate the mgm. dyestuff found in the urine and referred to 100 cc. glomerular filtrate. It appears that at about 10 mgm. per cent the rate of dyestuff transfer reaches a constant maximum value. It is suggestive to picture this result as being due to the shift of some carriers transporting a full load to the inner surface of the tubular

cells, where the carriers are discharged. Certainly, this is not more than a simple sketch as designed and discussed already in the older work of A. Schmidt, v. Sobieranski, Gurwitsch and others in their interpretation of the microscopic picture which appears after an injection of dyestuff and which, with certain dyestuffs, can display a regular arrangement of dyestuff granules along the inner border line of the epithelia. Here, the discussion shall be restricted to the following two remarks: First, the shape of the curve is like an adsorption isotherme. It says that, with low concentrations of phenol red being available for transfer, the carriers would not get the full load, but, the relative load would be greatest near zero concentration and would decrease more and more towards the higher ones. In other words, the accumulation ratio diminishes with rising concentration. This has been found frequently, e.g. as mentioned already before regarding the uptake of inorganic ions by the potato discs and by other plant tissues. Also it has been found in studying the reabsorption of glucose and the secretion of creatinine and others. The similar behavior of such a variety of substances makes one cautious against generalizing the hypothesis that adsorption plays an essential role in the transfer across the cells even though it sounds reasonable in the special case of dyestuffs. The second remark points to the fact that, during the transport of one of the acid dyes, under physiological conditions of the acting cells, no morphological structures are detectable with a high power microscope which could be interpreted as vehicles. Even the hepatic cells, while delivering into the bile ducts a secretion containing 1000 times more dyestuff than the perfusion fluid, do not show any colored structure. As a matter of fact, it is indicative of abnormal circumstances, when colored vacuoles appear during the active transfer of dyes (14, 26).

So far, in the secretion of dyestuffs, adsorption at the interface between the cell and its aqueous surroundings or at intracellular interfaces has been emphasized in this lecture to be an especially important factor. However, certainly such a structural organization is supplemented by a chemical organization, when, instead or apart of the secondary van der Waals valencies of adsorption, chemical main valencies are active in the transfer. For example, such an action can be attributed to some enzymes in considering their special placement in the cell body. Phosphorylation and dephosphorylation seem to be governing factors in the transport of sugar in the kidney. Following the work of Gomori, Kritzler and Gutman and Lipman, this assumption is supported not only by the location of the appartaining enzymes in the cortex of the kidney (27), but particularly by the fact that phosphatase is seated near the brush border of the epithelia (28, 29). Thus, sugar can be picked up at the cell entrance for further transport towards the capillaries surrounding the tubules. Similarly, carbonic anhydrase, which, according to Davenport, likewise is present in the cortex of the kidney, probably is an accelerator of the reabsorption of bicarbonate (30). By observations like those, light is being thrown upon the unidirectional passage of dissolved substances through cell layers, so frequently met in absorptive and secretory processes. The unilateral location of enzymes appears to be one factor in what has been called by Chambers the functional polarity of units involved in the active transfer (31).

Concluding, I wish to turn your attention once more to one of the most striking examples of active transfer, the simultaneous transfer of the cations and the anions of the neutral inorganic salts, so conspicuous in the experiments with plant tissues, when K ion and Cl ion or Rb ion and Br ion simultaneously are accumulated, and also with animals, e.g. in the reabsorption of Na and Cl by the frog kidney, in their absorption by the frog skin (11) and in the intestinal absorption of Na and Cl, known for a long time and more thoroughly studied by Ingraham and Visscher (12). Taking into account that, with inorganic ions, we are dealing with chemically rather inactive substances, it is suggestive to believe that the transfer somehow is due to electrical forces.

Now, from the work of Michaelis, Söllner, Mond, Wilbrandt and others (32), we know about the potentials seated in porous membranes. Their direction is determined by the positive or negative charge of the pore walls and by the properties of the free ions present. One can assume that circuit currents are flowing in one or the other direction and that they are transporting ions across the pores. Furthermore, considering the thickness of the surface membrane of cells to be not more than about 100 Angstrom units, the potential fall across the membranes will amount to 100 Volt and more. In addition, we know that, according to older and to the more recent investigations (33–37), in absorbing as well as in secreting membranes, the shift of the cations and the anions appears to be related to the amount of metabolism and to the height of potentials being located in the living membranes.

Taking together these various factors, it does not seem to go so very much astray to believe that, first, with artificial mosaic membranes provided with different pores, some selectively permeable to cations and others to anions (38, 39, 40), second, with a continuous supply of metabolic ions

(H^+ and HCO_3^-) and third with sufficiently high membrane potentials, the natural phenomenon of active transfer of neutral salts could be imitated (41, 42).

REFERENCES

(1) Amberson, W. R. and R. Höber. J. Cell. Comp. Physiol. **2:** 201, 1932. (2) Haywood, C. and R. Höber. J. Cell. Comp. Physiol. **10:** 305, 1937. (3) Höber, R. and P. Briscoe-Woolley. J. Cell. Comp. Physiol. **15:** 35, 1940. (4) Richards, A. N. and J. B. Barnwell. Proc. Roy. Soc., London, **B 102:** 72, 1927. (5) Höber, R. Cold Spring Harbor Symp. **8:** 40, 1940. (6) Höber, R., P. Briscoe-Wooley, J. Green and M. Zimmerman. J. Cell. Comp. Physiol. **19:** 183, 1942. (7) Höber, R. and A. Titajew. Pflüger's Arch. **223:** 180, 1929. (8) Höber, R. J. Gen. Physiol. **23:** 185, 1939. (9) Hoagland, D. R. and T. C. Broyer. Plant Physiol. **11:** 471, 1936. (10) Steward, F. C. Protoplasma **15, 16, 17, 18,** 1932–33. (11) Krogh, A. Skand. Arch. Physiol. **76:** 60, 1937. (12) Visscher, M. B. and R. C. Ingraham. Am. J. Physiol. **121:** 771, 1938. (13) Davenport, H. W. and R. B. Fisher. Amer. J. Physiol. **131:** 165, 1940. (14) Chambers, R. and R. Kempton. J. Cell. Comp. Physiol. **3:** 131, 1933. (15) Beck, L. V. and R. Chambers. J. Cell. Comp. Physiol. **6:** 441, 1935. (16) Koll-Schröder, M. Pflüger's Arch. **234:** 264, 1934. (17) Ferrari, R. and R. Höber. Pflüger's Arch. **232:** 299, 1933. (18) Collander, R. and A. Holmström. Acta Soc. Fenn. **60:** 129, 1937. (19) Hoagland, D. R. and A. R. Davis. J. Gen. Physiol. **5:** 629, 1923. (20) Hoagland, D. R. and A. R. Davis. J. Gen. Physiol. **6:** 47, 1924. (21) Marshall, E. K. and M. M. Crane. Am. J. Physiol. **70:** 465, 1924. (22) Scheminsky, F. Pflüger's Arch. **221:** 641, 1929. (23) Verzar, F. Biochem. Ztschr. **276:** 17, 1935. (24) Donhoffer, S. Arch. Exper. Path. und Pharmakol. **177:** 689, 1935. (25) Shannon, J. A. Physiol. Rev. **19:** 63, 1929. (26) Höber, R. J. Cell. Comp. Physiol. **6:** 117, 1935. (27) Gomori, G. Proc. Soc. Exper. Biol. Med. **42:** 23, 1939. (28) Kritzler, R. A. and A. B. Gutman. Am. J. Physiol. **134:** 94, 1941. (29) Lipman. Enzymology **1:** 99, 1941. (30) Höber, R. Proc. Soc. Exper. Biol. Med. **49:** 87, 1941. (31) Chambers, R. Cold Spring Harbor Symp. **8:** 144, 1940. (32) Höber, R. Physiol. Rev. **16:** 52, 1936. (33) Lund, E. J. J. Exper. Zool. **51:** 291, 1928. (34) Francis, W. L. J. Exper. Biol. **11:** 35, 1934. (35) Taylor, A. B. J. Cell. Comp. Physiol. **7:** 1, 1935. (36) Huf, C. Pflüger's Arch. **235:** 655, 1935. (37) Huf, C. Pflüger's Arch. **236:** 1, 1935. (38) Höber, R. and F. Hoffmann. Pflüger's Arch. **220:** 558, 1928. (39) Söllner, K. Biochem. Ztschr. **244:** 371, 1932. (40) Brooks, S. C. Protoplasma **8:** 389, 1929. (41) See the discussion about properties and functions of membranes. Trans. Faraday Society **33:** 911, 1937. (42) Wilbrandt, W. Ergebn. Physiol. **40:** 204, 1938.

THE SECRETION OF DYSTUFFS BY THE GASTRIC GLANDS AND THE PANCREAS

MAURICE B. VISSCHER

Division of Physiology in the University of Minnesota

A knowledge of the structural characteristics of the secreting mechanisms of the glands in the animal organism is obviously important to an understanding of the processes of secretion. The character of the materials which are transported across physiological barriers gives some information as to the nature of the barrier. Dyestuffs have been employed in studies upon numerous physiological barriers because they 1, provide a large variety of chemical configuration; 2, have representatives, some of which dissociate as acids and some as bases, with a great range of dissociation constants; 3, possess a wide range of solubility properties; 4, are subject to easy quantitative assay; and 5, present a spread of particle sizes into the colloidal range. Furthermore, it is generally believed that at low concentrations the dyestuffs have few important specific chemical effects on physiological processes.

The appearance or non-appearance of any solute, present in blood plasma, in a secretion may obviously depend upon a number of factors. Secreting structures consist of epithelial cells whose activities are responsible for the translocation of materials which are either derived as such from the blood or are elaborated by the cells, and appear in the gland lumen as the secretion. Water and inorganic materials are all necessarily derived from the blood. Certain organic materials are produced by the secreting cells. Other organic materials which find their way into the secretion come from the blood. The dyestuffs belong in

this category. It seems apparent that if any regularities can be found in the character of the dyestuffs which do and do not appear in one or another secretion, light will be thrown on the structural and functional characteristics of the gland in question.

The deductions which can be drawn from any such observations are, however, limited in several ways. It is not by any means certain that all of the components in a given secretion arrive by the same physical routes. Certain components of secretions, as for example enzymes, may very possibly be extruded as granules or in vacuoles by the secreting cells. Other components such as water and electrolytes may reach the gland lumen by other mechanisms or routes. Information obtained on water transport in protozoa (13) indicates that under certain conditions water turnover at rates equal to a thousand times the total cell volume per day may occur through the extrusion of vacuoles. Krogh (14) has pointed out that in the case of fresh water forms the vacuole fluid must be nearly pure water. Thus it seems likely that living cells possess the capacity to move water against large osmotic gradients by the vacuole formation and discharge mechanism. It is also equally possible, however, that the magnitude of concentration difference which can be set up between protoplasm and vacuole, can also be set up between protoplasm and intraluminal fluid. In view of the current lack of information as to the routes and mechanisms of material transport in secreting structures it is impossible to ascribe the ability of any substance to appear in a secretion to any definite functional structure in the gland. The most that can be said in general at the present time is that if certain classes of substances appear in a given secretion, and others do not, there must be a fundamental correlation between the properties distinguishing the two classes of substances and some characteristics of the gland or its secretion. The facts, for example, that a large variety of both acid and basic dyes of subcolloidal particle size appear in the glomerular fluid and that a number of colloidal dyes do not, allow the generalization that molecular size is a determinant for passage, and that electro-chemical characteristics are not determinants in this situation.

In order for any foreign material to appear in a secretion it must be able to permeate the effective barriers separating the gland lumen from the blood containing the foreign material. Furthermore the distribution ratios for the material must be such that appreciable quantities of the foreign material can be present in the secretion at equilibrium with the blood. This becomes important in the case of acids and bases in secretions with hydrogen ion activities considerably removed from that of blood. These are conditions with which we are actually concerned in the case of gastric and pancreatic juices. Further discussion of these points will be reserved until a later section of this review.

Microscopic observations upon the gastric mucosa and pancreas. The microscopic appearance of the living gastric mucosa in frogs following injection of various dyes has been described by Henning (15). He observed diffuse staining of the fundus region with several dyes, and reported the secretion of a number of both acid and basic dyes by the frog's stomach. Among the acid dyes he reported several colloidal dyes to be secreted. These are generally not found to be secreted in mammalian gastric juice and it is therefore doubtful whether the observations are pertinent to the problem in higher forms. Microscopic studies of the living mammalian gastric mucosa under histamine stimulation both with and without dye administration have recently been made (16) using the dog under amytal anesthesia. Light was transmitted from a 500 W. 110 V. bulb, using the bent quartz rod technique of Knisely. The stomach wall with blood supply intact was trans-illuminated by fixation with pins on a rigidly fixed thin cork ring fitted with two glass coverslips 3 mm. apart, between which Ringer's fluid at 37° C. was passed to prevent temperature changes. Direct contact of the quartz rod light source with the tissues results in damaging heating. Such preparations can be studied over a period of several hours. The secretion of dyes can be observed directly in this way in order to ascertain whether the fluid emerging from the gastric crypts contains secreted dyes, or whether the latter reach the secretion after the gastric juice flows over the surface of the mucosa by diffusion from the surface cells.

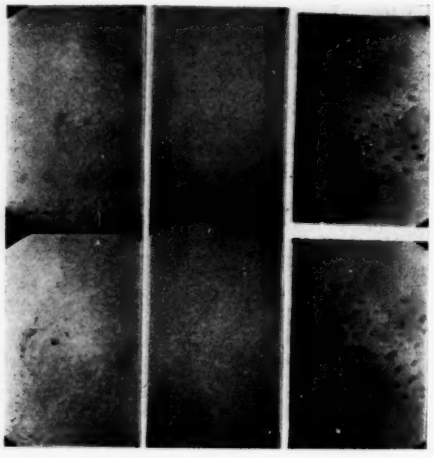
Figure 1a shows four photomicrographs of dog fundic mucosa at 100 diameters without dye administration. The mosaic pattern of the mucosa is evident, with an array of elliptical pore openings which constitute the orifices of the gastric crypts. In figure 1b are shown comparable photomicrographs after intravenous injection of methylene blue, 20 mgm. per kgm., during the secretory state induced by histamine. It will be evident that the elliptical pores are now darkly shadowed. The photographs are inadequate to show what is evident on direct microscopic study, namely that these dark shadows in the crypt orifices are microscopic droplets of gastric juice highly colored with dye.

It seems from such observations as these that the dyes which appear in gastric juice are present in the secretion as it leaves the crypt orifices, and are not derived by transfer through surface cells.

Such studies have not as yet thrown light on the

question of where in the gastric crypt the dyestuffs enter. Since the proportions of parietal and chief cells at different depths of the crypt are variable, information might be obtained by insertion of micro-pipettes and collection of fluid at different levels under histamine stimulation. This procedure has been performed for the measurement of electrical potentials, but not for collection of fluid. The desirability and feasibility of micromanipulative study of the structure and behavior of the secreting mammalian gastric mucosa needs emphasis. Methods are now available for such studies and much may be learned about the gastric secretory mechanism by their application.

The staining of gastric mucosa and the pancreas in dogs has also been studied by the frozen section technique applied to those tissues after intravenous administration of dyes of various classes. Werch (17) found, as will be evident from the results shown in table 1, that there is considerable regularity in the staining of these tissues by various classes of dyes. No acid dyes stain the pancreatic parenchyma, while a number of them pigment the gastric mucosa. Microscopic study showed that the chief cells were more deeply stained than other mucosal elements. As will be noted later the acid dyes are secreted by the pancreas, and not by the stomach. Thus there is an inverse relation between secretion and staining of stomach and pancreas with acid dyes. The acinar cells of the pancreatic parenchyma are deeply pigmented by basic dyes which that organ does not allow to pass into its secretion. In the case of certain amphoteric dyes the pancreas is obviously slightly stained, but the concentration of pigment was too small to be visible in microscopic study. In the case of the gastric mucosa many basic dyes produce staining, although others do not. Neutral red, for example, which is readily secreted by the gastric glands, causes pigmentation only of surface cells, which might occur secondarily by absorption. On the other hand a number of basic dyes stain the parietal cells moderately. These observations give no such clear basis for generalization as was found in the case of acid dyes.



1a 1b

Fig. 1a. Photomicrograph of living gastric fundic mucosa of dog.

Fig. 1b. Appearance of secreting gastric mucosa following intravenous injection of methylene blue.

Several limitations in such histological studies need to be borne in mind. Although tissues can be frozen nearly instantaneously and sectioned in that state, they are allowed to thaw before microscopic study. Movement of dye can and probably does occur in the injured and dying tissues. Total absence of staining is probably highly significant. Thus the results with acid versus basic dyes in pancreatic parenchyma probably have importance. The presence of basic dyes in the gastric mucosa is also undoubtedly real, but the significance of the site of staining at the time of observation is in doubt.

Studies on gastric pouches. Several groups of investigators have studied the appearance of dyes in gastric juice. It would be unprofitable to review the entire literature because a majority of the contributions deal with the secretion of one or a very few dyes and are of importance mainly to clinical problems. Dawson and Ivy (1), Kobayashi (2), Ingraham and Visscher (3), Morrison, Reeves and Gardner (4), and Varco and Visscher (5) have studied the appearance of rather large numbers of dyes of various classes in gastric pouch juice after their intravenous administra- in dogs. The conditions of observation were different in the several series of studies, but excepting the studies of Morrison et al., the results are in remarkable agreement.

The list of dyestuffs which have been found by several groups of workers to be present in gastric secretion from stomach pouches in the dog, following intravenous injection, is shown in table 2. It will be seen that examples of nine chemical classes of dyes appear in this list. There is thus no evidence of any specificity on this score. Studies on solubility characteristics, not shown in the table, but presented by several of the authors referred to, prove that there is no relation between oil-water distribution coefficients of various dyes and their ability to be secreted. Likewise there is no connection between their diffusion coefficients and their ability to be secreted (2). The only obvious regularity in the series has to do with electrochemical properties. It will be noted that all but one of the twenty-seven dyes listed are ordinarily classed as basic. Furthermore, in the case of Fuchsin S electrochemical studies,

which will be discussed more fully later, show this dye to be amphoteric in its reduced form. Its chromogen can therefore be present partially in cationic form. So in this series of dyestuffs there is no dye which is not electro-positive under some conditions.

Table 3 presents a partial list of the dyes which have been studied for secretion in gastric juice and have generally been found not to appear. This list shows, first, that many acid dyes of various classes have been studied, giving significance to the appearance of basic dyes as shown in table 2. The data in table 3 also show that one group of observers (4) found seven acid dyes to appear in gastric pouch juice. It is significant that no other observer has found any of these seven dyes to be so secreted, and that as many as three other groups of observers have studied the same dyes. Several of these acid dyes are colloidal in character and one, Congo red, is widely used in blood volume studies because of the slowness with which it leaves the blood. The present

TABLE 1

Staining of gastric mucosa and pancreas by intravenously injected dyes in the dog

DYES	COLOR INDEX NO.	STOMACH		PANCREAS	
		Gross	Microscopic	Gross	Microscopic
Group 1: Acid dyes not secreted by pancreas					
Fast wool blue B	209	Mucosa, submucosa and adventitia +++	Chief cells of mucosa, submucosa and adventitia +++	Parenchyma negative	Intima and adventitia of blood vessels +
Alizarine red S	1034	Negative	Negative	Parenchyma negative	Parenchyma negative
Erythrosine B	773	Negative	Negative	Parenchyma negative	Parenchyma negative
Trypan red	438	Negative	Negative	Parenchyma negative	Parenchyma negative
Group 2: Acid dyes secreted by pancreas					
Indigo disulphonate	1180	Mucosa, submucosa and adventitia +	Negative	Parenchyma negative	Parenchyma negative
Superchrome violet B	169	Negative	Negative	Parenchyma negative	Parenchyma negative
Alpha azurine 2 G	712	Mucosa, submucosa and adventitia ++	Chief cells of mucosa, submucosa and adventitia +	Parenchyma negative	Parenchyma negative
Orange I	150	Mucosa, submucosa and adventitia +	Negative	Parenchyma negative	Intima and adventitia of blood vessels +
Wool blue CG	59	Mucosa, submucosa and adventitia ++	Chief cells of mucosa, submucosa and adventitia ++	Parenchyma negative	Parenchyma negative
Group 3: Basic dyes secreted by stomach					
Neutral red	825	Negative	Surface cells +	Parenchyma ++	Acinar cells +
Methylene blue	922	Lower ⅔ of mucosa ++	Parietal cells +	Parenchyma +++	Acinar cells +
Methylene green	924	Lower ⅔ of mucosa ++	Parietal cells +	Parenchyma +++	Acinar cells +
Pyronine B	741	Lower ⅔ of mucosa ++	Parietal cells +	Parenchyma ++	Acinar cells +
Brilliant cresyl blue	877	Lower ⅔ of mucosa ++	Parietal cells +	Parenchyma ++	Acinar cells +
Group 4: Amphoteric dyes secreted by both organs					
Rhodamine B	749	Mucosa, submucosa and adventitia +	Negative	Parenchyma +	Parenchyma negative
Acridine red	740	Mucosa, submucosa and adventitia +	Negative	Parenchyma +	Parenchyma negative
Safranine O	841	Mucosa, submucosa and adventitia +	Negative	Parenchyma +	Parenchyma negative
Group 5: Basic dyes not secreted by stomach					
Methyl violet 2B	680	Lower ⅔ of mucosa ++	Parietal cells +	Parenchyma ++	Acinar cells +
Fuchsin Y	677	Lower ⅔ of mucosa ++	Parietal cells +	Parenchyma ++	Acinar cells +

author has tested the secretion of Congo red in seven dogs with surgically prepared gastric pouches and has never found it to appear in histamine-excited gastric juice after intravenous administration of the dye. This and other acid dyes appear in serous exudates, and it seems possible that the disagreement in results may have been due to the use of incompletely healed gastric pouches in the series of experiments in which these acid dyes appeared. The complete disagreement with all other observers would seem to prove that some such adventitious factor vitiates the results in question, as far as the problem at hand is concerned. It is obvious that only basic and amphoteric dyes are reproducibly found to be secreted in gastric pouch juice.

Studies on pancreatic secretion. The appearance of intravenously injected dyestuffs in pancreatic juice in the dog, excited by secretin, pilocarpine or ingestion of food, has been studied by several workers. The collected results of four such studies are presented in table 4. Only the thirty-five dyes found to be secreted are listed. More than fifty other dyes were tested, including equal numbers of basic and acid dyestuffs from all the main chemical categories. Of the thirty-five substances secreted, twenty-five are conventionally classed as acid dyes. Of the remaining ten, only six have been studied electrochemically as to migration in an electric field. These are all found to be amphoteric under some conditions. The remaining four are chemically closely related to dyes known to be amphoteric, and are therefore likely to be so also.

There is no demonstrable relationship between solubility characteristics or chemical groups and secretion capacity for these substances. The only observable regularity is that for all of the dyes which have been so studied (thirty-one of the thirty-five secreted) the chromogen moves as

TABLE 2

Dyestuffs eliminated by the gastric glands

DYE	COLOR INDEX NO.	CHEMICAL CLASSIFICATION	APPLICATION CLASS	LOCATION OF CHROMOGEN	APPEARANCE IN GASTRIC JUICE IN CONCENTRATION RELATIVE TO INJECTED BLOOD CONCENTRATION WHERE MEASURED			
					1*	2*	3*	4*
Chrysoidine Y	33	Azo	Basic	In cation	+	+	75.0	+
Janus Black		Azo	Basic			+		
Bismark brown	331	Dis azo	Basic	In cation	+	+	26.5	+
Fuchsin S	692	Triphenylmethane	Acid	Anion when oxidized, both when reduced	+	−	0.5	
Malachite green	657	Triphenylmethane	Basic	In cation	+		+	
Rhodamine B	749	Pyronine	Basic	Both anion and cation	+		6.1	
Pyronine B	741	Pyronine	Basic	In cation			5.7	
Acridine red	740	Pyronine	Basic	Both anion and cation			8.4	
Indophenol	821	Indophenol	Basic	In cation			+	
Methylene green	924	Thiazine	Basic	In cation			13.6	
Methylene blue	922	Thiazine	Basic	In cation†	+	+	15.6	+
Thionin	920	Thiazine	Basic	In cation	+	+	36.8	+
Toluidin blue	925	Thiazine	Basic	In cation	+		36.4	+
Azure I		Thiazine	Basic			+		
Azure II	923	Thiazine	Basic	In cation		+	+	+
Navy blue (S.B.)		Thiazine	Basic			+		
Nile blue B	913	Oxazine	Basic	In cation	+		0.8	
Brilliant cresyl-blue	877	Oxazine	Basic	In cation			11.3	
Cresyl echtviolet		Oxazine	Basic	In cation			3.4	
Naphthol blue-chloride		Oxazine	Basic		+			
Cyanamine bichloride		Oxazine	Basic		+			
New blue D 120		Oxazine	Basic			+		
Neutral red	825	Azine	Basic	In cation	+	+	26.4	+
Neutral violet		Azine	Basic	In cation			27.2	+
Safranin O	841	Azine	Basic	Cation when oxidized, both when reduced		+	27.3	+
Safranin T extra		Azine	Basic			+		
Thioflavine T	815	Thiazol	Basic	In cation			1.3	

*Numbers refer to authors cited in bibliography.

†Partially amphoteric in the reduced form at pH 8.4.

an anion under appropriate conditions. The deduction has been drawn (3) that the pancreas secretes in general only electronegative chromogens.

The electrochemical properties of dyestuffs. A great deal of physiological work has been done employing dyes in permeation and accumulation studies. The chemical literature does not provide adequate information about the electrochemical characteristics of these substances over the physiologically significant pH range, and for the reduced as well as the oxidized state for reversibly reducible dyes. Such studies have been carried out for the list of dyes employed in gastric and pancreatic secretion studies by Ingraham and Visscher (3). It seems desirable to present in this connection their previously unpublished results with those dyestuffs which behave anomalously, in order to clarify previous references to the subject in this paper. The method employed was the study of migration of dyes in oxidized or reduced form at various hydrogen ion concentrations. The dyes were studied in 0.05 per cent solution buffered at the values listed. Reduction

TABLE 3

Dyestuffs not eliminated according to most observers

DYE	COLOR INDEX NO.	CHEMICAL CLASSIFICATION	APPLICATION CLASS	LOCATION OF CHROMOGEN	APPEARANCE IN GASTRIC JUICE				
					1*	2*	3*	4*	5*
Naphthol yellow	9	Nitro	Acid	In anion			−		
Resorcin green	1	Nitroso	Acid	In anion			−		
Naphthol green	14	Nitroso	Acid	In anion			−		
Fast wool blue B	209	Azo	Acid	In anion			−		
Wool orange 2G	27	Azo	Acid	In anion			−		
Wool scarlet BR	280	Azo	Acid	In anion			−		
Methyl orange	142	Azo	Acid	In anion			−		
Orange I	150	Azo	Acid	In anion			−		
Superchrome violet B	169	Azo	Acid	In anion			−		
Wool blue CG	59	Azo	Acid	In anion			−		
Fast fuchsin 6B	56	Azo	Acid	In anion			−		
Methyl red	211	Azo	Acid	In anion			−		
Orange G		Azo	Acid					+	−
Congo Red		Azo	Acid	In anion	−	−		+	−
Trypan red	438	Dis Azo	Acid	In anion		−	−	+	−
Trypan blue	477	Dis Azo	Acid	In anion	−	−	−		
Brilliant blue CFC	855	Azine	Acid	In anion			−		
Wool fast blue BL	833	Azine	Acid	In anion			−		
Light green yellowish SF	670	Triphenylmethane	Acid	In anion			−		
Water blue	706	Triphenylmethane	Acid	In anion		−	−		
Brilliant green	662	Triphenylmethane	Basic	In cation			−		
Methyl violet 2B	680	Triphenylmethane	Basic	In cation			−		
Pyrrol blue		Triphenylmethane	Acid	In anion		−	−		
Alpha azurine 2G	712	Triphenylmethane	Acid	In anion			−		
Rosolic acid	724	Triphenylmethane	Acid	In anion			−		
Gentian violet	680	Triphenylmethane	Basic	Cation when oxidized, both when reduced			−		
Fuchsin Y	677	Triphenylmethane	Basic	Cation when oxidized, both when reduced			−		
Alizarin red S	1034	Oxyketone	Acid	In anion			−		
Alizarin sapphire	1054	Oxyketone	Acid	In anion			−		
Erythrosine B	773	Pyronine	Acid	In anion		−	−	+	−
Rose bengal	777	Pyronine	Acid	In anion			−	+	−
Brom phenol blue		Pyronine	Acid	In anion			−		
Brom thymol blue		Pyronine	Acid	In anion			−		
Uranine yellow	766	Pyronine	Acid	In anion		−	−		
Acid-fast violet GRF	758	Pyronine	Acid	In anion					
Eosin BS	771	Pyronine	Acid	In anion		−	−	+	−
Phenol red		Pyronine	Acid	In anion			−		
Phenolphthalein	764	Pyronine	Acid	In anion		−	−		
Cresol red		Pyronine	Acid	In anion			−		
O-Cl-phenol indophenol		Indophenol	Acid	In anion			−		
Thioflavine G	815	Thiazol	Acid	In anion			−		
Cochineal powder	1239	Natural	Acid	In anion		−	−		
Lithium carmine		Natural	Acid			−		+	−
Indigo disulfonate	1180	Natural	Acid	In anion			−		

* Numbers refer to authors cited in bibliography.

was accomplished either with dilute sodium hydrosulfite or with hydrogen and platinized asbestos. Reoxidation was performed by 1 per cent hydrogen peroxide. Solutions of reduced dyes were covered with mineral oil to prevent significant entry of oxygen. The results obtained with a group of anomalous dyes are presented in table 5. It will

Furthermore reduced methylene blue behaves as though a portion of this substance were a weak acid at pH 8.4. These observations serve to demonstrate the necessity for study of the electrochemical properties of dyestuffs employed in physiological investigation. It is obviously entirely unsatisfactory to accept the technical

TABLE 4

Dye secretion by the pancreas

DYE	COLOR INDEX NO.	CHEMICAL CLASSIFICATION	APPLICATION CLASS	LOCATION OF CHROMOGEN	APPEARANCE IN PANCREATIC JUICE IN CONCENTRATION PER CENT OF INJECTED BLOOD CONCENTRATION WHERE MEASURED			
					3*	6*	7*	8*
Resorcin green	1	Nitroso	Acid	In anion	5.1			
Naphthol yellow	9	Nitro	Acid	In anion	0.2			
Martius yellow	6	Nitro	Acid	—†				+
Methyl orange	142	Azo	Acid	In anion	0.1	+		
Orange I	150	Azo	Acid	In anion	0.4			
Superchrome violet B	169	Azo	Acid	In anion	0.5			
Wool blue CG	59	Azo	Acid	In anion	0.7			
Fast fuchsin 6B	56	Azo	Acid	In anion	0.6			
Methyl red	211	Azo	Acid	In anion	1.6			
Amaranth	168	Azo	Acid	—				+
Fast yellow	16	Azo	Acid	—		+		
Alpha azurine 2G	712	Triphenylmethane	Acid	In anion	0.2			
Rosolic acid	724	Triphenylmethane	Acid	In anion	0.2			
Fuchsin S	692	Triphenylmethane	Acid	Anion when oxidized, both when reduced	0.2	+		
Gentian violet	680	Triphenylmethane	Basic	Cation when oxidized, both when reduced	0.1			
Fuchsin Y	677	Triphenylmethane	Basic	Cation when oxidized, both when reduced	0.7			
Para fuchsin	676	Triphenylmethane	Basic	—		0.9		
Basic fuchsin	678	Triphenylmethane	Basic	—		+		
Methyl violet	515	Triphenylmethane	Basic	—				+
Uranine yellow	766	Pyronine	Acid	In anion	0.5	+	+	
Acid-fast violet GRF	758	Pyronine	Acid	In anion	0.5			
Eosin BS	771	Pyronine	Acid	In anion	+			
Eosin AG	—	Pyronine	Acid	—			+	
Eosin W yellow	—	Pyronine	Acid	—			+	
Phenol red	—	Pyronine	Acid	In anion	+			
Phenolphthalein	764	Pyronine	Acid	In anion	0.3			
Cresol red	—	Pyronine	Acid	In anion	0.2			
Acridine red	740	Pyronine	Basic	Both anion and cation	1.8			
Rhodamine B	749	Pyronine	Basic	Both anion and cation	1.4	6.5		
Brilliant blue CFC	855	Azine	Acid	In anion	0.4			
Wool fast blue BL	833	Azine	Acid	In anion	0.1			
Safranin O	841	Azine	Basic	Cation when oxidized, both when reduced	0.4	+		
Safranin extra bluish	842	Azine	Basic	—		0.1		
Methylene blue	922	Thiazine	Basic	Cation when oxidized, both at pH 8.4 reduced		+		
Indigo disulfonate	1180	Natural	Acid	In anion	1.0			

* Numbers refer to authors cited in bibliography.

† The mark — indicates that no studies of migration at various pH values and in the reduced and oxidized state are available.

be noted that Acridine red and Rhodamine B, which are ordinarily classed as basic dyes, are in reality amphoteric mixtures in the oxidized form. In the reduced form Rhodamine B behaves as a basic dye at pH 1.2 and 5.0 and as an acid one at pH 8.4. The first five dyes in the table all behave as amphoteric mixtures in the reduced form.

application class as evidence of acid or basic characteristics under all circumstances. Further more the notorious irregularity of dyes of different origins and batches and the existence of mixtures of related but different substances in a given sample, make the study of each preparation employed essential. Finally the change in electro-

chemical characters upon reduction is crucial in biological studies. Many dyes are reversibly reduced in blood or body cells. In these studies it was found that Pyronine B and Indophenol are secreted in gastric juice in completely reduced form. Reoxidation was necessary for their detection. As a routine all colorless samples of secretions studied were treated with an oxidant before being called negative. The fact that certain dyes were secreted in reduced form by the gastric glands suggests that further studies on the oxidation-reduction characteristics of the secreting structures with these methods might be fruitful.

No extensive study has been made of the electrochemical behavior of dyestuffs in physiological fluids such as blood. The degree of association with proteins or other components of such systems may play an important role in determining their behavior.

TABLE 5

Dye ion migration in an electric field

mm./ 30 min. P.D. 2 V./cm.

DYE	STATE	TO ANODE				TO CATHODE				SECRETION
		pH 1.2	pH 5.0	pH 7.3	pH 8.4	pH 1.2	pH 5.0	pH 7.3	pH 8.4	
Safranin O	Oxidized	0	0	0	0	3	4	1	1	G. and P.*
	Reduced	3	7	4	5	5	15	5	7	
Fuchsin S	Oxidized	7	4	10	5	0	0	0	0	G. and P.
	Reduced	15	19	21	13	9	2	1	0	
Fuchsin Y	Oxidized	0	0	0	0	2	9	4	5	P.
	Reduced	4	11	8	12	17	10	3	3	
Gentian Violet	Oxidized	0	0	0	0	3	3	2	4	P.
	Reduced	2	4	6	5	6	3	2	0	
Malachite Green	Oxidized	0	0	0	0	7	5	6	3	G.
	Reduced	1	9	9	3	5	4	1	0	
Acradine Red	Oxidized	8	3	2	1	3	6	2	4	G. and P.
Rhodamine B	Oxidized	3	4	6	7	5	6	3	4	G. and P.
	Reduced	0	0	0	1	4	3	0	0	
Methylene Blue	Oxidized	0	0	0	0	5	5	4	7	G. and P.
	Reduced	0	0	0	1	6	13	6	6	

* G. indicates secretion in gastric juice; P. in pancreatic juice.

The absorption of dyes from the stomach. The ability of dyes to penetrate from the lumen of the stomach to the blood is of some consequence to the interpretation of secretion studies. If the same classes of dyes move in the two directions it might be deduced that surface cells offered the effective barrier to movement. On the other hand if there were no such correlation the specificity of the secretory mechanism becomes more probable. The data obtained by Kobayashi (9) in such studies using 0.5 per cent dye solutions in the stomach are presented in table 6. It will be apparent that both acid and basic dyes are absorbed from the stomach and that there is a correlation between diffusibility in agar gels, as measured by that author, and the ability to be absorbed. Further studies showed no connection between lipoid solubility and absorption.

The fact that there is no electrochemical selectivity with respect to movement of dyestuffs from gastric lumen to blood appears to strengthen the support for the conclusion that such a selectivity in regard to movement in the opposite direction results from some corresponding electrochemical specificity in the structures responsible for the secretion of gastric juice.

General discussion and conclusions. With the reservations discussed previously it is concluded that present evidence indicates that only those dyestuffs whose chromogen can be positively charged either in the oxidized or reduced form appear in gastric juice. For the pancreatic juice, for all dyes for which electrochemical information is available, it appears that the chromogen must be able to occur as an anion in the oxidized or reduced forms in order to be able to be secreted.

Such a situation might result from corresponding electrochemical specificity in the barriers between blood and gland lumen which mediate the secretion process. A filter with ion specificity could produce such a result. It is perhaps not without importance that electronegative artificial

membranes restrain the passage of electropositive dyes, and vice versa (3). If such an effect were responsible for the observed results it would imply that certain structures through which materials passed in the gastric secretory process acted as electronegative filters. It might easily be that only undissociated dye molecules passed such filters, and that the reason for the non-

TABLE 6

Absorption of dyes from the stomach

Kobayashi (9)

	CLASS	DIFFUSIBILITY	ABSORPTION
(1) Monoazo			
1. Chromotrope 2R	Acid	8.5	(+)
2. Chrysoidine cryst	Basic	8.5	(+)
3. New Phosphine G patent	Basic	8.0	(+)
4. Ponceau R	Acid	7.5	(+)
5. Scarlatina R	Acid	7.5	(+)
6. Azo Fuchsine G	Acid	7.0	(+)
7. Azo Rubine S	Acid	5.5	(+)
8. Janus Black I	Basic	0-3.0	(−)
(2) Disazo			
1. Bismarck Brown XXX	Basic	7.0	(+)
2. Trypan Blue	Acid	6.0	(+)
3. Congo Red	Acid	4.5	(−)
4. Benzo Azurine G 386	Acid	0-1.5	(−)
5. Azo Blue	Acid	0-1.2	(−)
(3) Triphenylmethane			
1. Fuchsine S	Acid	7.5	(+)
2. Magenta cryst	Basic	5.0	(−)
3. Aniline Blue (water soluble)	Acid	4.5	(−)
4. Hofmann's Violet	Basic	1.5(−4.5)	(−)
5. Water Blue	Acid	3.5	(−)
(4) Xanthene			
1. Phenolsulphonephthalein	Acid	8.5	(+)
2. Uranine	Acid	8.0	(+)
3. Phloxine NBB	Acid	7.5	(+)
4. Eosine AG	Acid	7.0	(+)
5. Acridine Red	Basic	0-1.5	(−)
(5) Azine			
1. Navy Blue No. 1918	Basic	5.0	(−)
2. Neutral Red	Basic	3.5	(−)
3. New Blue D 120	Basic	3.0	(−)
4. Azur I	Basic	2.0	(−)
5. Methylene Blue conc. BB	Basic	2.0	(−)
(6) Other groups			
1. Indigocarmine la opt	Acid	6.5	(+)
2. Haube B Fluorescent	Basic	6.5	(+)
3. Lithiumcarmine	Acid	4.5	(−)

passage of dyes which dissociate with an opposite charge is that they are fixed instantaneously upon dissociation and only undetectable quantities pass the full length of a pore without dissociation. If some phenomenon such as this did not occur one would expect that electronegative dyes would be restrained by similarly charged membranes, as the ions of strong electrolytes are known to be (10). By invoking the theory that dyes enter and pass through the pores of charged membranes in undissociated form it becomes possible to harmonize the information we have on dyestuff permeation with the well-established facts regarding permeation of strong electrolytes.

Developing this line of reasoning further it would follow that the effective barriers in the path of gastric gland secretion would be electropositive and therefore relatively impermeable to cations of strong electrolytes. The low sodium and high hydrogen ion concentrations of gastric juice as compared with blood almost require that such a situation exist at the surfaces of the gland cells with the lumen if the latter cell membranes are not virtually semipermeable. At least it is difficult to understand how dynamic factors alone could account for the maintenance of the high concentration gradients which exist for the ions mentioned. It is, of course, not a necessary conclusion that the secreting structures themselves have such permeability characteristics.

There is another possible type of explanation of the observed results. When two phases of different hydrogen ion concentration contain a salt of a weak acid or base and are separated by a third phase in which the undissociated acid or base is soluble, a distribution difference will be set up. The result will be that basic dyes will accumulate in the more acid phase and acid dyes in the more alkaline. This situation has been discussed by Irwin (11) and by Osterhout (12). This effect produces concentration differences of larger magnitude the greater the dissociation constant of the weak acid or base. Ingraham and Visscher (3) calculated that for dyes with dissociation constants smaller than 1×10^{-5} this effect would be too small to account for the distribution differences actually observed. Since many of the dyes have much smaller dissociation constants it seems unlikely that this otherwise attractive hypothesis will account for the known facts in relation to dye secretion by acid and base secreting glands.

The main facts at hand concerning the secretion of dyestuffs in gastric and pancreatic juice point to an interpretation on the basis of an electrochemical structural specificity at some important barriers in the pathways of secretion in those glands. It is impossible to arrive at more than suggestive deductions because the rest of our information about the secretory processes involved is too fragmentary. Nevertheless the dye studies point to certain plausible structural characteristics, a consideration of which may be useful in further studies on the more intimate mechanism of secretion in these glands.

REFERENCES

(1) DAWSON, A. B. AND A. C. IVY. Am. J. Physiol. **73:** 304, 1925. (2) KOBAYASHI, K. Kioto Univ. Acta Scholae Medicinalis **8:** 465, 1926. (3) INGRAHAM, R. C. AND M. B. VISSCHER. J. Gen. Physiol. **18:** 695, 1935. (4) MORRISON, S., D. L. REEVES AND R. E. GARDNER. Am. J. Digestive Dis. and Nutrition **3:** 551, 1936–37. (5) VARCO, R. L. AND M. B. VISSCHER. Proc. Soc. Exper. Biol. and Med. **46:** 295, 1941. (6) CRANDALL, L. A., E. OLDBERG AND A. C. IVY. Am. J. Physiol. **89:** 223, 1929. (7) MATSUO, I. Kioto Univ. Acta Scholae Medicinalis **10:** 4, 1928. (8) ZIEGLER, M. R. AND L. B. MENDEL. Am. J. Physiol. **82:** 299, 1927. (9) KABAYASHI, K. In Biologische Untersuchungen Uber Farbstoffe by I. Matsuo, Kyoto, 1935, pp. 848–858. (10) MICHAELIS, L. J. Gen. Physiol. **8:** 33, 1925. (11) IRWIN, M. Proc. Soc. Exper. Biol. and Med. **26:** 125, 1928. (12) OSTERHOUT, W. J. V. Ergebn. Physiol. **35:** 967, 1933. (13) KITCHING, J. A. J. Exper. Biol. **11:** 364, 1934; **13:** 11, 1936; **15:** 143, 1938. (14) KROGH, A. Osmotic regulation in aquatic animals. Cambridge, 1939, p. 23. (15) HENNING, N. Arch. f. Exper. Path. u. Pharmakol. **165:** 191, 1932. (16) GELFAN, S. AND M. B. VISSCHER. Unpublished observations. (17) WERCH, S. C. A histo-physiologic study of the stomach and pancreas. Thesis, University of Illinois, 1936.

THE FORMATION OF ACID BY THE GASTRIC GLANDS

JOHN S. GRAY

Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago

The mechanism which enables the gastric glands to form a secretion 3 million times more acid than the blood which nourishes them is nearly as great a mystery today as it was a century ago. This fascinating problem has repeatedly challenged theorists to attempt its solution, usually with results which might charitably be described as disappointing. Because so little was known about the physiology of the gastric glands, it was unfortunately considered that only one process needed explaining—how hydrochloric acid can be formed from other chemical compounds. Thus the approach was frequently that of elementary chemistry rather than that of biochemistry or physiology and the resulting theories were nonviable, if not still-born.

Perhaps we can avoid this sterile approach to the problem of acid formation if we first consider the facts which are now known about the behavior of the gastric glands and then judge some of the newer theories in the light of these facts. As a "come-on," it might be disclosed in advance that such an approach does lead to worthwhile conclusions about the mechanism of acid formation.

It is generally accepted that the acid in gastric juice is formed by the parietal cell, which is one of three types of cell found in the gastric glands. Accordingly, when we speak of acid formation we are referring to the secretory activity of the parietal cell.

One of the first steps in gaining an understanding of the activity of a gland is to determine exactly what the gland secretes. For example, the mechanism of formation of the glomerular filtrate was clearly revealed by a quantitative analysis of the glomerular filtrate. In the case of the secretion of the parietal cell the situation is more complicated because it has not yet been possible to collect pure parietal secretion for analysis. The difficulty is not insurmountable, however, for the methods employed by Lim (1), Hollander (2), and ourselves (3) permit quite reliable estimates of the composition of the parietal secretion. These methods are based on the fact that each of the three types of gastric gland cell secretes one characteristic and identifying substance; the parietal cells secrete acid, the chief cells pepsin, and the mucous cells, mucin. It is therefore possible to identify elements secreted by the parietal cell by virtue of the fact that their concentration in gastric juice must vary with the acidity. In like fashion the elements secreted by the chief cells and mucous cells must vary with the pepsin and mucin, respectively. A complete anlysis of this type has not yet been made, but the substances secreted by the parietal cell have been fairly well identified and distinguished from those secreted by the other cells (3). Figure 1 shows the results obtained when the concentrations of three representative constituents of canine gastric juice are plotted against the acidity. Substances not secreted by the parietal cell exhibit a negative slope in the graph. The sodium line is the prototype, but included in this category are nitrogen compounds, calcium, phosphorus, and in fact most other constituents of gastric juice. The sole element which exhibits a positive slope is the chloride ion, which must, therefore, originate mainly in the parietal cell. Potassium is unique, in that its concentra-

tion in gastric juice is constant, which indicates that it is secreted in the same concentration by all the cells, including the parietal cells. By further analysis of data of this type it is possible to determine the exact concentration of the various ions in the parietal secretion. The procedure is too technical to explain in the short time available, but the results show that the parietal secretion in the dog contains 166 m.eq./l. of chloride ion, 7 of potassium ion, and 159 of hydrogen ion. This solution is isotonic with dog's blood.

Hydrochloric acid in a concentration of 159 m.eq./l. is a fairly strong acid, having a pH below one, and one may feel obliged to point out that it has not been possible to demonstrate any such acidity in the lumen of the gastric glands by the

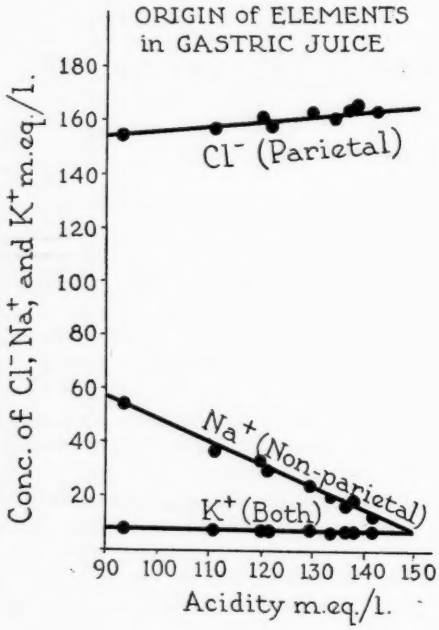


FIG. 1

method of supravital staining with dye indicators (4, 5). However, the almost insuperable difficulties which beset attempts to determine the intraluminal pH during actual secretion, render negative results of little significance. This is readily appreciated when it is remembered that dye indicators are not overly reliable, that differential rates of diffusion of hydrogen ions and organic dyes must inevitably distort the picture, and, most important of all, that the parietal cells cease to secrete immediately upon death of the animal or upon removal of gland tissue for examination. Furthermore, there is still some uncertainty as to whether the indicators are actually secreted by the parietal cell (6).

On the basis of this type of experiment, it has been suggested that the parietal secretion is neutral, as secreted, and becomes acid only upon reaching the surface of the gastric mucosa. If this development of acidity is accomplished by the absorption of water, it would require the absorption of one million liters of water for every liter of gastric juice finally formed. The absorption of water at this rate would tax even the kidney tubule, and the gastric gland tubule is lined by cells which appear to have only secretory functions. To assume that the acid is secreted in combined form does not circumvent the difficulty, because there is nothing in gastric juice which could perform this function and reabsorption of such an hypothetical substance would present a formidable problem.

Since gastric juice has usually been found to be isotonic, it has been suggested that water is actually absorbed in the gastric tubule until isotonicity is reached (7). However, gastric juice is frequently hypotonic to an extent exactly predicted by the loss of CO_2 which results from the reaction of acid parietal secretion with alkaline mucous secretion, as shown by Visscher (8) and ourselves (3). Since some of this interaction probably occurs within the gland tubule, the hypotonicity of gastric juice is evidence against water reabsorption.

In view of these observations it is felt that until contrary evidence is forthcoming, one is justified in assuming that the parietal secretion is an isotonic solution containing principally hydrochloric acid.

There are other pertinent facts which investigations of the past few years have revealed concerning the behavior of the parietal cells. In 1930 Delrue (9) reported that if the gastric mucosa is carefully dissected from a frog's stomach and then mounted between two small artificial baths, the membrane would form acid, as revealed by a fall of pH in the solution on the secretory side of the membrane. In extending these observations we have shown that this acid formation is a true secretory process and not a post-mortem phenomenon, nor a non-specific, semi-permeable membrane effect (10). Further experiments unexpectedly revealed that secretion was not prevented by the omission of bicarbonate or phosphate ions from the nutrient bath solution (11). These results indicate that the acid forming cells do not depend upon an external source of phosphate or bicarbonate ions for the secretion of acid.

Davenport (12) has recently contributed another important observation concerning the behavior of the parietal cells. He has shown that these cells extract chloride or bromide ions from the blood, depending upon their concentration in that medium, with complete impartiality.

Careful analysis of the process revealed convincingly that physical properties only, and not chemical properties, determined their selection from the blood. This constituted a striking demonstration of the fact that the parietal cells are concerned particularly with the secretion of hydrogen ions, and that chloride ions accompany the former passively, as the most readily available anions of the blood.

There have been several attempts to attack the problem of acid formation by studying the changes which occur in the blood during its passage through the stomach. Such studies have been meager and most of them have been incomplete in important aspects. They have, however, established several important facts which we will have occasion to consider in some detail later. There is still a need for careful and complete studies of this nature.

Having established a few facts concerning the behavior of the parietal cells, we are now in a position to examine certain theories which have been proposed to explain the process of acid formation. It would profit us little to examine all the theories. In fact, there is only one theory which is promising enough to warrant detailed discussion. This is the carbonic anhydrase theory proposed by Davenport. Carbonic anhydrase is the enzyme which accelerates the hydration of CO_2 and the dehydration of carbonic acid, whose presence and function in the red blood cell have been so ably investigated by Roughton and others.

In 1938 Davenport and Fisher (13) discovered that carbonic anhydrase is present in the gastric mucosa of a variety of animal species. Further investigation (14, 15) revealed that nearly all of the enzyme is located within the parietal cell and in concentrations as great or greater than in the red blood cell. The method used for localizing the enzyme in the parietal cell was a modification of the Linderstrom-Lang technique, which has been used to localize a number of substances in the cells of the gastric mucosa (16, 17, 18). This is a technique worth describing. In principle, it consists of taking tiny pieces of gastric mucosa from various regions and various depths in the mucosa and correlating the enzyme distribution with cell distribution. In more detail, discs of tissue are punched out of the gastric membrane. Each of these discs is cut into sections parallel to the mucosal surface. The enzyme concentration is then determined on the first and third of consecutive sections and cell counts are made on the intervening section. By plotting the enzyme analyses against cell counts one can determine which cell contains the enzyme. By this procedure Davenport found the correlation between parietal cell distribution and carbonic anhydrase distribution to be very high.

This work demonstrated rather conclusively that the parietal cell contains considerable quantities of carbonic anhydrase. What is the significance of this finding? The possible role of carbonic anhydrase in the formation of acid becomes apparent upon consideration of the behavior of the parietal cell. You will recall that the parietal secretion contains 166 m.eq./l. of chloride, whereas the plasma contains only 110 m.eq./l. Thus the parietal cell extracts large quantities of chloride from the blood. It is quite impossible to withdraw only a negative ion from the blood, leaving behind a solution unbalanced in its electrical charges. If the principle of electrical neutrality is not to be violated, there are only three ways in which the parietal cell can obtain the necessary chloride ions. *First*, the chloride may be transferred in un-ionized and thus in uncharged form as an organic chloride. But this is unlikely, for Brunschwig (19) has shown that radioactive chloride ion on intravenous injection can be detected in the gastric juice within 60 seconds. *Second*, the chloride ion may be transferred accompanied by an equivalent number of positive ions, such as hydrogen ion. There are several objections to this possibility, among which is the consequence that carbonic anhydrase in the parietal cell becomes completely superfluous, a scientific curiosity and nothing more. *Third*, the chloride ions removed from the blood may be replaced by an equivalent number of some other negative ions, such as bicarbonate ion, which could be supplied by the parietal cell. In order to provide an inexhaustable supply of bicarbonate ions, the parietal cell must convert its continuously produced carbon dioxide immediately into carbonic acid, a process requiring the services within the parietal cell of the enzyme, carbonic anhydrase. Hence it is at once apparent that if chloride is obtained from the blood by anion exchange, carbonic anhydrase in the parietal cell serves an eminently useful purpose. If the parietal cell contributes to the blood the bicarbonate moiety of the carbonic acid, what becomes of the hydrogen moiety? It cannot also be sent into the blood, because this would upset the electrical balance achieved by exchanging chloride for bicarbonate. Obviously this hydrogen ion, made available in quantities equivalent to that of chloride obtained from the blood, would serve admirably for completing the hydrochloric acid which is needed for the parietal secretion.

On *a priori* grounds we have thus arrived at the conclusion that the parietal cell, by virtue of the carbonic anhydrase which it contains, does not yield up to the blood carbon dioxide gas or carbonic acid, but bicarbonate ions only. If this conclusion is valid, gastric venous blood must differ in certain predictable and essential ways

from other venous blood. Thus by searching for these differences, the *a priori* conclusions can be put to the acid test of experimental observation.

Just what are these differences? First, blood passing through the gastric glands must exhibit a reversed chloride shift. You will remember that when blood passes through most body tissues, muscle for example, carbon dioxide gas, not bicarbonate ions, enter the plasma from the tissues. The gas diffuses into the red blood cell, where it is hydrated to carbonic acid by the carbonic anhydrase. After dissociation, the bicarbonate ions diffuse back into the plasma and an equivalent number of chloride ions diffuse *into* the red cell to replace them. This is the normal chloride shift. In the case of blood passing through the gastric glands, on the other hand, it has been predicted that bicarbonate ions, not carbon dioxide gas, will enter the plasma from the tissues. These bicarbonate ions will diffuse into

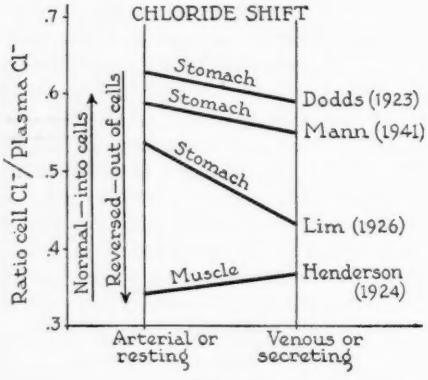


FIG. 2

the red cell, and an equivalent number of chloride ions will diffuse *out of* the red blood cell. This is the reversed chloride shift. You will note that the direction of the chloride shift depends upon whether carbon dioxide gas, or bicarbonate ions are contributed to the blood by the tissues; the entrance of carbon dioxide causes a normal shift of chloride into the red cells and the entrance of bicarbonate ions causes a reversed shift of chloride out of the red cells. I have compiled some average figures from data available in the literature which illustrates the reversed chloride shift in gastric blood. The full significance of these data were apparently not appreciated by the original authors. Davenport (20) was the first to point out the necessity of the reversed shift. Figure 2 shows the changes in cell-to-plasma ratios of chloride in blood before and after passing through muscle tissue and stomach. You will note that the line labeled "muscle" has a positive slope, indicating that chloride is entering the red cells according to the normal shift (21). The other lines referring to blood passing through the stomach exhibit a negative slope, indicating that chloride is leaving the red cell, according to the reversed chloride shift. The data recently reported by Mann et al. (22) represent true arteriovenous differences, but the other two "stomach" lines represent systemic blood taken first during rest and then during active gastric secretion (23, 24). It may be concluded from these data, that in accordance with prediction, the parietal cell must be contributing bicarbonate ion rather than carbon dioxide gas to the blood.

On the basis of the *a priori* conclusion it was also predicted that the parietal cell must contribute its bicarbonate ion to the blood, unaccompanied by hydrogen ions. Hence the second difference between gastric and systemic venous blood must be a difference in pH. Blood passing through muscle tissue becomes more acid as a result of the entrance of carbon dioxide which is hydrated to carbonic acid. Blood passing through the stomach, on the other hand, must become more alkaline, if bicarbonate ions are added in exchange for chloride ions. It has been observed by Hanke et al. (25) that the blood does actually show an increase in pH on passing through the actively secreting stomach. Because the ratio of carbonic acid to bicarbonate in blood is 1 to 20, the addition of one equivalent of carbonic acid will acidify blood to a greater degree than one equivalent of bicarbonate will alkalinize it. Therefore, since the blood actually does become alkaline, it must mean that all or nearly all of the carbon dioxide formed within the parietal cell must be converted to carbonic acid and none or nearly none of the resulting hydrogen ions gain access to the blood; they must be diverted to the parietal secretion. A corollary to this conclusion is that the parietal cell forms no more carbon dioxide than is required to provide the hydrogen ion content of its secretion. If an excess were formed, the blood would not be rendered alkaline on passing through the stomach.

The available experimental evidence, therefore, supports the view that the carbonic anhydrase within the parietal cell is functional.

The carbonic anhydrase theory of acid formation states that the carbon dioxide formed within the parietal cell by its metabolic activity, is converted immediately into carbonic acid by the activity of the enzyme, carbonic anhydrase. The acid then ionizes and the resulting hydrogen ions are diverted into the parietal secretion. At the same time the bicarbonate ions are sent into the blood in exchange for an equivalent amount of chloride ions, which are then diverted into the secretion to complete the hydrochloric acid. This theory accounts for the presence of carbonic

anhydrase in the parietal cell; it accounts for the reversed chloride shift and the alkalinity of gastric venous blood with its accompanying alkaline tide; it accounts for the independence of the parietal cell on external sources of carbon dioxide; it is in harmony with the passive nature of chloride secretion. In these respects the carbonic anhydrase theory is a major advance over previous theories. On the other hand, the theory is admittedly an incomplete explanation of the mechanism of acid formation. It is not concerned with the most baffling problem of all, namely, the mechanism by which the hydrogen ions are concentrated to an isotonic strength against such a formidable concentration gradient.

This discussion of the carbonic anhydrase theory would not be complete without considering another aspect of the problem about which there has been some disagreement. Carbonic anhydrase is readily inhibited by a number of compounds, such as thiocyanate and sulfanilamide, which are reasonably well tolerated by the animal organism. If it could be shown that these drugs also inhibit gastric secretion, it would provide further evidence in favor of the carbonic anhydrase theory. Davenport (26) has investigated this possibility and has shown that thiocyanate reduces the acidity of gastric juice obtained from dogs injected with histamine. He believed at first that this inhibition was exerted through the action of the drug on the gastric carbonic anhydrase. However, he subsequently showed (20) that only partial inactivation of the highly concentrated enzyme in the cell could be expected from the attainable thiocyanate concentrations. Furthermore, if what Roughton et al. (27) have recently demonstrated for the red blood cell holds also for the parietal cell, the amount of carbonic anhydrase present is in excess of requirements by as much as 100 times. Hence nearly complete inactivation of the enzyme would be required to produce inhibition of secretion. Accordingly Davenport now believes that some other explanation must be sought for the inhibitory effect of thiocyanate on gastric secretion.

Sulfanilamide is even more potent than thiocyanate in inhibiting carbonic anhydrase. Davenport (28) has accordingly investigated its effects on gastric secretion. He has found that in high concentrations a mild degree of inhibition of secretion is obtained, and that this is all that could be expected in view of the circumstances mentioned above.

Feldberg, Keilin, and Mann (29) on the basis of a few acute experiments reported that thiocyanate inhibited gastric secretion, whereas sulfanilamide failed to do so. They stated, without including the data in their tables, that the sulfanilamide completely inhibited the gastric carbonic anhydrase, whereas the thiocyanate did not inhibit. At first glance these data appear to disprove the carbonic anhydrase theory, but I do not believe that this is the only interpretation that can be made of them.

In summary, the study of carbonic anhydrase inhibitors has not so far yielded any evidence which either strongly supports or denies the carbonic anhydrase theory of acid formation. Other evidence known at present is distinctly favorable to the theory. Of course, new evidence may swing the balance one way or the other in the future, but whether the theory is eventually proved or rejected, it must be said of it that it stimulated more productive investigation into the mechanism of acid formation than any previous theory.

So far in this discussion nothing has been said about the energy exchange involved in the secretion of acid by the parietal cell. Since the concentrations of the various ions in both the plasma and the parietal secretion are known, it is possible to calculate on a thermodynamic basis the minimum free energy required to concentrate the ions in parietal secretion. The results of such calculations applied to our data by Bull (30) are shown in table 1. It is apparent that most of the energy is applied to the hydrogen ions which are more than 3 million times more concentrated in the juice than in the plasma. These calculations show that a minimum of 1500 small calories is required to form one liter of parietal secretion. As a basis for comparison it might be mentioned that Borsook and Winegarden (31) have similarly calculated that about 700 small calories are required to form one liter of urine.

TABLE 1

Free energy required to form 1 liter of parietal secretion

ION	PLASMA CONC., M.	PARIETAL CONC., M	FREE ENERGY
			gm. cal.
H^+	4×10^{-8}	0.159	−1488.0
Cl^-	0.110	0.166	−42.1
K^+	0.005	0.0074	−1.8
Total			−1531.9

What is the source of this energy? It cannot be filtration pressure of the blood, for Bull (30) has calculated that the blood pressure would have to be at least 5000 mm. of Hg in order to provide the necessary energy! It is probable, although not certain, that the parietal cell, like the salivary glands (32), depend mainly upon glucose oxidation for the energy needed for secretion. We have found that the isolated gastric mucosa of the frog fails to form even traces of acid in the

presence of cyanide, which inhibits oxidation in general, or iodoacetate, which inhibits glucose oxidation in particular (33). We have not as yet had the opportunity to study the effects of other enzyme inhibitors.

Let us assume, for lack of more certain information, that glucose oxidation provides the energy for the parietal cell and that the end product of oxidation is carbon dioxide from which the secreted hydrogen ions are obtained. In order to supply the 159 m.eq. of hydrogen ions contained in one liter of parietal secretion, approximately 4.8 grams of glucose must be oxidized. It has already been pointed out that the parietal cell cannot form excess carbon dioxide, otherwise the gastric venous blood would not become alkaline. Hence it is of interest to know whether the oxidation of 4.8 grams of glucose would provide the 1.5 large calories of energy needed to form the liter of secretion. If not, it might be a serious objection to our previous conclusions. The amount of free energy released by the oxidation of 4.8 grams of glucose cannot be calculated exactly, because the conditions in the parietal cell are not fully known. But it would appear that sufficient energy would be made available, even after allowing for the considerable inefficiency in the utilization of energy which characterizes biological reactions.

A final question now arises and it is by far the most imposing one of all. How does the parietal cell utilize its energy to produce such an amazing concentration of hydrogen ions? One might suggest that carbonic acid produced within the cell accumulates until its concentration is high enough to provide the necessary hydrogen ion concentration. Unfortunately for this suggestion Davenport (14) has calculated that the necessary carbonic acid concentration would be 17 molar! This is obviously absurd, but it is quite possible that the carbonic acid concentration within the cell exceeds that in the plasma, and hence motivates the chloride-bicarbonate exchange. But this would provide traces of acid, not an isotonic concentration, and we are again confronted with the problem of explaining how the cell concentrates the acid.

We know what the parietal cell secretes; we know where it gets the raw materials and how it gets the raw materials; we know how much energy is required and where it gets the energy; but we still do not know how the cell uses this energy to concentrate its secretory products against such imposing concentration gradients. This problem is not peculiar to the physiology of the parietal cell; it is repeatedly encountered in other fields. The absorption of ions by the intestine against concentration gradients is still a mysterious process. The conversion of energy liberated by chemical reactions to mechanical work by muscle cells is equally mysterious. It is possible that this general problem in physiology may find its first solution as a result of continued investigation into the mechanism of acid secretion by the stomach.

REFERENCES

(1) Liu, A. C., I. C. Yuan and R. K. S. Lim. Chin. J. Physiol. **8:** 1, 1934. (2) Hollander, F. Am. J. Digest. Dis. **5:** 364, 1938. (3) Gray, J. S. and G. R. Bucher. Am. J. Physiol. **133:** 542, 1941. (4) Hoerr, N. L. and R. R. Bensley. Anat. Rec. **65:** 417, 1936. (5) Dawson, A. B. and A. C. Ivy. Am. J. Physiol. **76:** 158, 1926. (6) Lerner, H. H., L. Asher and K. Andrews. Am. J. Digest. Dis. **9:** 109, 1942. (7) Gilman, A. and G. R. Cowgill. Am. J. Physiol. **103:** 143, 1933. (8) Lifson, N., R. L. Varco and M. B. Visscher. Proc. Soc. Exper. Biol. and Med. **47:** 422, 1941. (9) Delrue, G. Arch. Inter. de Physiol. **33:** 196, 1930. (10) Gray, J. S., J. L. Adkison and K. Zelle. Am. J. Physiol. **130:** 327, 1940. (11) Gray, J. S. and J. L. Adkison. Am. J. Physiol. **134:** 27, 1941. (12) Davenport, H. W. and R. B. Fisher. Am. J. Physiol. **131:** 165, 1940. (13) Davenport, H. W. and R. B. Fisher. J. Physiol. **94:** 16P, 1938. (14) Davenport, H. W. J. Physiol. **97:** 32, 1939. (15) Davenport, H. W. Am. J. Physiol. **128:** 725, 1940. (16) Holter, H. and K. Linderstrom-Lang. Ztschr. f. physiol. chem. **226:** 149, 1934. (17) Linderstrom-Lang, K. and A. S. Ohlsen. Enzymologia **1:** 92, 1936. (18) Glick, D. J. Gen. Physiol. **21:** 297, 1938. (19) Brunschwig, A. and R. L. Schmitz. Proc. Soc. Exper. Biol. and Med. **43:** 438, 1940. (20) Davenport, H. W. Personal communication. (21) Henderson, L. J, A. V. Bock, H. Field and J. L. Stoddard. J. Biol. Chem. **59:** 379, 1924. (22) Mann, F. D., J. H. Grindley and F. C. Mann. Am. J. Digest. Dis. **8:** 451, 1941. (23) Dodds, E. C. and K. S. Smith. J. Physiol. **58:** 157, 1923. (24) Lim, R. K. S. and T. G. Ni. Am. J. Physiol. **75:** 475, 1926. (25) Hanke, M. E., R. E. Johannesen and M. M. Hanke. Proc. Soc. Exper. Biol. and Med. **28:** 698, 1931. (26) Davenport, H. W. Am. J. Physiol. **129:** 505, 1940. (27) Roughton, F. J. W., D. B. Dill, R. C. Darling, A. Graybiel, C. A. Knehr and J. H. Talbott. Am. J. Physiol. **135:** 77, 1941. (28) Davenport, H. W. Am. J. Physiol. **133:** P257, 1941. (29) Feldberg, W., D. Keilin and J. Mann. Nature **146:** 651, 1940. (30) Bull, H. B. and J. S. Gray. Unpublished. (31) Borsook, H. and H. M. Winegarden. Proc. Nat. Acad. Sci. **17:** 3, 1931. (32) Deutsch, W. and H. S. Raper. J. Physiol. **92:** 439, 1938. (33) Ivy, H. B. and J. S. Gray. Unpublished.

THE EFFECT OF NERVOUS AND HORMONAL STIMULATION ON ZYMOGEN SECRETION

J. EARL THOMAS

Jefferson Medical College of Philadelphia

The secretion of enzymes takes place in two stages. The first is the synthesis and storage in the cell in the form of zymogen granules of materials which are later to appear in the secretion as enzymes. The second is the discharge of stored zymogen material from the cells into the secretion. The first is a continuous activity of the unstimulated cell (Von Anrep and Kahn, 1924). The second process is discontinuous and occurs only in response to specific stimuli reaching the cells by way of the nervous system, or through the blood stream in the form of specific hormones. The present discussion is concerned exclusively with this latter activity. Even so it will be possible in the time available to make only a superficial survey of the problem within definite limits. I shall consider only the salivary glands, the peptic glands of the stomach and the pancreas.

THE SALIVARY GLANDS. *Nervous control.* The principal facts regarding the innervation of the salivary glands are too well known to require repetition. The literature has been reviewed by Pavlov (1907) and more recently by Babkin (1914, 1926, 1938a) and by Stormont (1932).

Stimulation of the peripheral secretory nerves with an electric current or by means of appropriate drugs brings about secretion of saliva containing enzymes, usually measured as total organic matter, in amounts depending upon the nerve stimulated, the strength and duration of the stimulus and the condition of the gland. Parasympathetic stimulation in most animals produces a copious flow of saliva which contains a variable but relatively small amount of organic matter. Sympathetic stimulation (except in cats) results in a more concentrated secretion. When the nerves are stimulated over a long period of time the enzyme concentration diminishes progressively and ultimately reaches very low levels due to exhaustion of the zymogen content of the cells. Strong stimulation usually results in a secretion having a higher content of organic matter than does weak stimulation. Provided the gland is not exhausted an increase in strength of the electrical stimulus results in a temporary increase in the enzyme content of the saliva (Heidenhain, 1878).

Reflexes affecting salivary secretion include a great variety of conditioned reflexes (Pavlov, 1904), reflexes from the mucosa and taste buds of the mouth and reflexes elicited by electrical stimulation of certain afferent nerves (see Babkin, 1914). The content of organic matter and presumably, therefore, the enzyme content of saliva secreted in response to reflex stimulation varies with the nature and the site of application of the stimulus used to initiate the reflex (Wulfson, 1898; Sellheim, 1904; Heymann, 1904).

Prolonged stimulation of the salivary glands through the efferent nerves or by means of reflexes (e.g., taking food) results in definite histological changes in the cells. This fact was first observed by Heidenhain (1868, 1878) and the character of the changes elucidated by Langley (1879). More recently the matter has been further studied by a large number of investigators. (For literature see Metzner, 1907 and Stormont, 1932.) The most striking and significant feature of the cytological change is the disappearance of zymogen granules from the secreting cells. According to Langley (1879) stimulation of either the sympathetic or parasympathetic nerves results in such changes in the cells. In some special instances, notably in the case of the sympathetic nerve supply to the dog's parotid gland, according to Heidenhain (1878), it is possible to bring about these changes without the production of any visible secretion.

Most of these facts have been known to investigators for many years and have given rise to a number of theories designed to explain them, especially the qualitative variations in the secretion. The earliest and best known is the theory of Heidenhain (1878) who postulated the existence of two kinds of excitatory nerves to the salivary glands. One kind he described as "secretory nerves" and ascribed to them the function of causing the secretion of water and inorganic salts. The nerves which promote the secretion of enzymes he described as "trophic" probably because he believed that they promoted the synthesis as well as the discharge of organic matter from the cells. Most of these theories, including that of Heidenhain, are obsolete as applied to the salivary glands but the ideas expressed by the terms "secretory" and "trophic" are still useful in discussing the diverse influences affecting the functions of secreting cells. The word "trophic" is misleading since it now has reference only to the discharge of zymogen material from the cells and Babkin (1938a) suggests the use in its place of the term "ecbolic" and as a substitute for the term "secretory" he proposes "hydrelatic." Babkin (1942) now believes that in general the "trophic" or "ecbolic" function is mediated by the parasympathetic nerves and not by the sympathetics as Heidenhain thought.

Heidenhain's theory was based on the assumption that each individual cell receives a double innervation. Stormont (1925) and, more recently, Rawlinson (1933, 1935 a and b, 1936) have shown that this is not the case; rather each individual type of cell receives its innervation from one source only. For example, according to Rawlinson, the mucous cells of the submaxillary gland of the cat are innervated exclusively by parasympathetic fiber whereas the demilune cells are innervated by the sympathetic. Assuming these interpretations to be correct, the qualitative variations of the salivary secretion present no special problem. Since the salivary glands contain different types of cells capable of secreting saliva containing a larger or smaller amount of organic matter or perhaps none at all, the variations in the character of the secretion may be referred to the predominating activity of one or another type of gland cell and this in turn to selective stimulation of the appropriate nerve fibers (Babkin, 1934b). In the case of the salivary glands at least, it is not necessary to assume that any single cell exhibits more than one characteristic type of functional activity, i.e., that it secretes more than one kind of saliva.

Hormonal control. With regard to the possibility of a humoral influence on the secretion of saliva, two observations are of interest. Ostrogorski (1894) observed that electrical stimulation of the sciatic nerve may cause secretion of saliva in the pilocarpinized cat after all the known efferent nerves to the salivary glands have been cut. This is probably, as suggested by Florovsky (1917), the result of secreted epinephrine. Babkin and his co-workers (1932) have shown that the denervated submaxillary gland in an eserinized cat will secrete on stimulation of the opposite undegenerated chorda typmpani nerve. We have repeated this observation on dogs as a classroom demonstration. So far as I know, there has been no study of the content of organic matter of the saliva produced by either of these procedures. While this may be called a humoral effect on the salivary gland, it is obviously dependent upon the now familiar neurohormones and is in no way analogous to the effect of specific secretory hormones such as are believed to influence the gastric glands and the pancreas. Efforts to demonstrate a specific secretory hormone for the salivary glands have given negative results (Sacks and Kim, 1929).

PEPSIN SECRETION BY THE GASTRIC GLANDS. *Nervous regulation.* Excitatory fibers for the gastric glands reach the stomach by way of the vagi and splanchnics. The gastric secretory fibers in the vagus nerves were discovered in 1888 by Pavlov and Simanowskaja (1888, 1895). They devised the esophageal fistula and the method of sham feeding and observed that after vagotomy sham feeding caused no secretion of gastric juice. They also observed that when suitable precautions were taken to avoid effects on the circulation, electrical stimulation of the peripheral vagus trunks caused a definite secretion of gastric juice. The important fact from our standpoint is that the gastric juice obtained in such experiments contains an extraordinarily high concentration of pepsin (Ouschakov, 1896). Toby (1936) has recently shown that sham feeding superimposed on histamine stimulation greatly increases the output of pepsin.

Direct evidence that the vagus fibers act on the chief cells of the gastric glands to bring about an outpouring of pepsin has been obtained by a study of the histological changes in these cells under the influence of vagus stimulation. That such changes are associated with pepsin secretion was proven by Langley and Sewall in 1879. Histological studies have recently been reported by Ishikawa (1929) and by Bowie (1936) and Bowie and Vineberg (1935). The latter authors demonstrated practically complete disappearance of pepsin granules from the chief cells of the gastric mucosa of dogs after 9 hours of continuous vagus stimulation.

It is commonly stated in the literature that the effect of stimulation of the sympathetic nerves (splanchnics) on the gastric secretion is questionable except for an increase in the secretion of mucus. However, it appears from the very careful work of Volborth and Kudryavzeff (1927) that if appropriate precautions are taken as was done by Pavlov in his studies of the vagus innervation, splanchnic stimulation may produce a secretion of acid gastric juice, though less than is obtained on vagus stimulation. Baxter (1932–1934) noted an increase in mucus secretion but no acid on stimulation of the splanchnic nerves in cats and dogs. The aplanchnics, however, probably have little effect on the pepsin content of the gastric juice (Baxter, 1932, 1934). In amphibians the sympathetic nervous system provides the chief secretory innervation of the stomach (Friedman, 1934).

The nervous mechanism is involved chiefly in the first or cephalic phase of gastric secretion during which the gland cells are stimulated, as in the case of the salivary glands, by a great variety of conditioned reflexes and by unconditioned reflexes from the mouth (Pavlov and Simanowskaja, 1888, 1895). That it may be involved also in the later, so-called chemical, phases of gastric secretion has often been suspected but the participation of a specific reflex arc never has been conclusively demonstrated (see Babkin, 1926, 1934a–1938b). Suggestive evidence lies in the fact that atropine depresses all phases of gastric secretion (Sanozki,

1893; Zeljony and Savitsch, 1911 and 1912; Klein, 1933, and others). The evidence is inconclusive because atropine depresses also gastric motility and may have some effect on absorption of secretagogues. The experiments of Tomaszevski (1918) are particularly interesting. He found that injection of Witte's peptone into the duodenum of a dog brought about a copious secretion of gastric juice after a latent period of about 20 minutes. No such response was obtained after double vagotomy. Tomaszevski concluded, on what seems to me to be inadequate evidence, that the secretory effects observed were the result of conditioned reflexes. A somewhat similar observation was made by Ivy and Javois (1925) who found that placing hydrolyzed protein in the dog's intestine induced a secretion of gastric juice, also after a latent period of about 20 minutes. This effect was abolished by atropine.

Hormonal regulation. One difficulty in discussing hormonal influences on the gastric secretion arises from the fact that not all investigators agree that there is a gastric secretory hormone and those who accept its existence are not yet in agreement on what it is. Sacks, Ivy, Burgess and Vandolah (1932) came to the conclusion that "Either histamine is the gastric hormone or, if not, there is no gastric hormone or the gastric hormone has never been extracted from the gastric mucosa." A similar conclusion had been reached previously by Rothlin and Gundlach (1921). Komorav (1938-1942), on the other hand, has isolated a substance from the pyloric mucosa which has a pronounced stimulating action on gastric secretion but which, on the basis of his evidence, could hardly be histamine. This substance which as yet has not been chemically identified may well be the long sought for "gastrin" of Edkins (1906). Its effects on gastric secretion so far as they have been investigated are similar to those of histamine. We may, therefore, be justified in assuming that the effects of histamine with respect to the secretion of pepsin by the gastric mucosa are fairly representative of those of the gastric secretory hormone, be it histamine or something else.

The question of whether or not histamine stimulates the secretion of pepsin by the chief cells of the gastric mucosa has not yet been definitely answered. Vineberg and Babkin (1931) and independently Gillman and Cowgill (1931) using a similar technic obtained secretion of gastric juice, following the administration of histamine, in which the peptic activity was extremely small and seemed to approach zero. They used a method similar to the so-called double histamine test in which two doses of histamine are given about one hour apart. The peptic activity of the gastric juice diminished progressively during the secretion following the first injection of histamine but because of the increased volume the total output of pepsin during this phase appeared to be increased. The second dose of histamine, however, brought about a secretion with a still lower pepsin content in which, in some of the experiments, even the increased volume of secretion failed to increase the total output. Pratt (1940) has reported the collection of gastric juice entirely devoid of peptic activity following histamine stimulation. The experiments so far mentioned were done on dogs and cats. Results with a similar significance were obtained in birds by Friedman (1939) and in humans by Toby (1937). The several authors mentioned agree in general with the conclusion of Vineberg and Babkin that "Histamine activates selectively those cytological elements of the gastric glands which secrete water, hydrochloric acid and other inorganic constituents without affecting enzyme secretion." This point of view is supported by the work of Alley (1935) from Babkin's laboratory who found that if histamine was administered during the secretion provoked by sham feeding in a dog the volume of the gastric juice was increased but the peptic power was diminished almost to the level of that provoked by histamine alone. She concluded that histamine while stimulating the parietal cells actually inhibits the action of the vagi on the peptic cells, preventing the discharge by them of zymogen granules.

The effect of prolonged histamine administration on the zymogen content of the peptic cells as observed microscopically has been studied by Lim and Ma (1926) and more recently by Bowie and Vineberg (1935). The former used pouch and fistula animals (dogs, cats, and rabbits) without anesthesia. They administered 0.2 mgm. per Kg. of histamine hourly for 24 hours and found that, "The zymogen content of the peptic cells" was "largely reduced by the eighth hour," after which the appearance of the cells remained "almost unchanged." They reported also that under these conditions "the output of pepsin continues for long periods not showing any notable depression until twelve or more hours have elapsed." Bowie and Vineberg used dogs anesthetized with chloralose and urethane. They administered histamine in the same hourly dosage but over a shorter period of time. They observed no change in the zymogen content of the peptic cells during a 4 to 8 hour period.

A considerable number of authors disagree with the conclusions of Vineberg and Babkin quoted above. These include Bloomfield (1932); Polland (1932); Polland and Bloomfield (1929); Blakely and Wilkinson (1933); Helmer, Fouts and Zerfas (1932); Meinberg and Brown (1933); and Bucher, Ivy and Gray (1941). Most of them have studied

human subjects in which satisfactory experimental conditions are difficult if not impossible to establish. The most convincing experiments are those of Bucher, Ivy and Gray which were made under controlled conditions on experimental animals. They found that when continuous secretion of gastric juice was elicited over a period of hours by frequent injections of small doses of histamine the pepsin content of the gastric juice was maintained at a fairly constant level. Furthermore, it was increased when the rate of secretion was increased by increasing the dose of histamine. Also Bucher and Ivy (1941) using the double histamine test on whole gastric pouches did not find the concentration of pepsin greatly diminished following the second dose of histamine.

A final conclusion is impossible at the present time. Nevertheless, it is evident that histamine or hormonal stimulation of the gastric glands, whatever the hormone may be, is far inferior to nerve stimulation as a means of promoting the secretion of pepsin.

Secretion of enzymes by the pancreas. The effect on pancreatic secretion of stimulation of peripheral nerves was first investigated by Heidenhain (1875) but without much success. The first clear demonstration of the existence of pancreatic secretory fibers in the vagus nerves was made by Pavlov (1893) who showed that the unsatisfactory results previously obtained had been due to the association of inhibitory with the excitatory fibers in the vagi and to the changes in blood circulation which ordinarily accompany peripheral vagus stimulation. By chronically exposing one vagus nerve in the neck, dividing it and then stimulating it several days later after the cardiac fibers had degenerated, he was able to show that electrical stimulation of the peripheral vagus consistently brought about a free flow of pancreatic juice.

Among the numerous investigators who have since confirmed Pavlov's observations we may mention Von Anrep (1914), Korovitsky (1923), Baxter (1931) who studied rabbits, and Crittenden and Ivy (1937) who found that stimulation of the vagi still promoted the secretion of pancreatic juice after removal of the entire small intestine, thus proving that the effect was not due to an increased output of secretin. An outstanding characteristic of the pancreatic secretion provoked by stimulation of the vagus nerves is the high content of organic matter, chiefly protein (Babkin and Savitsch, 1908) and the high enzyme content (Savitsch, 1909). The protein content, according to Babkin and Tichomirov (1909), closely parallels the enzyme activity. Harper and Vass (1941) confirmed Korovitsky's observation that vagus stimulation in cats does not increase the volume of pancreatic secretion but they found that it does increase the concentration of enzymes in secretion obtained by other means.

The splanchnic nerves also contain secretory fibers for the pancreas (Kubrewetsky, 1894; Babkin, Hebb and Sergeyeva, 1939). Most investigators agree that the splanchnic secretory fibers are paralyzed by atropine, hence they are cholinergic. Stimulation of the splanchnics in the rabbit increases both the volume and concentration of the pancreatic juice according to Baxter (1931) but Harper and Vass (1941) believe that the sympathetic nerves in the cat contain fibers which have a selective inhibitory influence on the secretion of enzymes.

Histological studies of the pancreas strongly support the inference that the vagus nerves exercise a selective influence on the secretion of enzymes. The fact that the acinar cells of the pancreas when in a resting condition contain large numbers of zymogen granules which tend to disappear during active secretion was observed by Heidenhain (1875) and confirmed by Kühne and Sheridan-Lea (1882). The effect of vagus nerve stimulation on the microscopic appearance of the gland was studied by Scaffidi (1907) and later but under more satisfactory conditions by Babkin, Rubaschkin and Savitsch (1909). These investigators found that in dogs prolonged stimulation of the vagus nerves brought about changes in the pancreas corresponding to those previously observed during secretory activity. The zymogen granules decreased in number and tended to accumulate near the acinar borders of the cells. These observations have recently been repeated on cats by Sergeyva (1938) who also studied the effects of stimulation of the splanchnic nerves. In her experiments sympathetic stimulation had an effect similar to that of the vagus except that it was less pronounced.

Very little is known about the mechanism through which the excitatory nerves to the pancreas are normally activated. There is a slight degree of stimulation of the pancreas during the act of eating which is analogous to the psychic or reflex phase of gastric secretion. The amount of pancreatic juice secreted under these conditions is small, amounting to only a few cubic centimeters, and can have little practical influence on digestion. Fats and soaps in the intestine produce a secretion of pancreatic juice which is very rich in enzymes and resembles that obtained on stimulation of the vagus nerves (for literature see Babkin, 1914). The amount and enzyme activity of this secretion is reduced by the administration of atropine (Bylina, 1913), hence it is probably, in part at least, a result of reflexes acting through the excitatory nerves.

The copious secretion which results from the entrance of acid into the small intestine is gener-

ally considered to be caused by secretin set free by the acid. However, some investigators (Fleig, 1903a and b; Wertheimer, 1901; Wertheimer and LePage, 1902; Pavlov, 1907) have suggested that there may be a nervous factor even in the response to acid. This idea is supported by the experiments of Bylina in 1913, who found that the administration of atropine diminished the volume and the tryptic activity of the pancreatic juice secreted in response to the continuous injection of acid into the small intestine. We have found that the volume of the secretion in response to acid is reduced by double cervical vagotomy (Crider and Thomas, unpublished experiments). We also found that HCl in the intestine brings about a slightly more concentrated secretion than does purified secretin. This result is opposite to that obtained long ago by Savitsch (1909) and by Babkin and Savitsch (1908), doubtless with crude preparations of secretin.

Other products of digestion likely to be present in the intestine are generally regarded as having little effect on pancreatic secretion. Carbohydrates in general are inert, although cane sugar was found by Babkin and Savitsch (1921) to increase the enzyme content of pancreatic juice stimulated by acid. Peptones in the intestine have been variously considered to have no direct effect on pancreatic secretion (Rasenkow, 1929; Gray, Kim and Ivy, 1936), to decrease the volume of the secretion obtained in response to acid (Frouin, 1907; Popielski, 1919) or to increase the enzyme content of the pancreatic juice secreted in response to other excitatory agents but to be ineffective alone (Brjuchanow, 1913).

In this connection some recent experiments by Thomas and Crider (1941) are of interest. We used a new method for collecting pancreatic juice in which there was no loss of secretion except during periods of observation. We, therefore, avoided the constant exposure of the intestinal mucosa to unneutralized gastric juice and the disturbances in acid base balance and intestinal digestion which are unavoidably associated with the usual type of pancreatic fistula. Under these conditions injection of a moderate amount of a solution containing products of protein digestion, for example, 20 cc. of a 5 per cent solution of a commercial peptone ("Bacto-protone") or a similar amount of a pepsin digest of casein regularly resulted in the secretion of from 1 to 4 cc. of pancreatic juice having a high specific gravity and a high nitrogen content. These experiments have been repeated on 12 animals without a single negative result. The possibility that the secretion might be due to gastric juice escaping into the intestine was excluded by the very short latent period, which is usually less than 3 minutes and by an efficient arrangement for draining away gastric secretion through a gastric fistula. The possibility of its being secondary to the entrance of bile or water into the intestine was eliminated by appropriate control experiments. Inert material injected in the same way was without effect, hence conditioned reflexes were not involved.

In 2 animals in which the vagus nerves were chronically exposed and paralyzed during the periods of observation by injection of cocaine or procaine into the nerve trunks, no response was obtained following the injection of peptone into the intestine so long as the vagi remained paralyzed (Crider and Thomas, 1941). A similarly negative result was obtained immediately following double cervical vagotomy. Such semiacute experiments are open to several sources of error and appropriate chronic experiments are now in progress.

In recent unpublished experiments conducted in collaboration with Dr. Ramsay the effect of prolonged stimulation of the pancreas by means of repeated injections of peptone into the intestine has been studied by histological methods. In unanesthetized animals with a portion of the pancreas made accessible under a skin flap so that specimens could be taken from time to time for histological study, the injection of 20 cc. of 5 per cent Bacto-protone every 10 minutes for 6 hours brought about changes in the appearance of the acinar cells corresponding very closely to those following several hours of vagus stimulation as found by Sergeyva (1938) and by Babkin, Rubaschkin and Savitsch (1909).

Hormonal control. There is room for considerable difference of opinion regarding the effect of secretin on the production of enzymes by the pancreas. The characteristic effect of repeated doses of secretin as observed by most investigators (Morel and Terroine, 1909; Lalou, 1910; Von Anrep, Lush and Palmer, 1925; Mellanby, 1925; Langstroth, McRae and Komarov, 1939) bears a superficial resemblance to the effects of histamine on the gastric glands. It produces a secretion which contains a fair amount of enzyme in the beginning but gradually becomes more and more dilute as the secretion progresses until it finally attains a constant concentration at a low level. However, Von Anrep, Lush and Palmer (1925) and more recently Barrington (1941) have found that the concentration of enzymes in the secretion could be increased by increasing the rate of secretion through the effect of increasing doses of secretin. This occurred even after the vagi were cut or atropine had been administered. This latter effect has been interpreted as indicating a specific excitatory effect of secretin on enzyme production. On the other hand, it could be the result of bringing into activity cells which had previously not been stimulated by the submaximal

concentrations of secretin used. Such cells, by analogy with the pancreas as a whole, would produce a more concentrated secretion for a time.

In this connection the observations of Babkin, Rubaschkin and Savitsch (1909) are of considerable interest. These authors found that prolonged activity induced by injecting acid into the intestine caused only minor changes in the microscopic appearance of the pancreatic cells. There was some decrease in the number of granules which the authors attributed to the tendency of the fluid passing through the cells to wash out a certain amount of zymogen material by mechanical action. They reached the conclusion that secretin has no specific influence on the secretion of enzymes by the pancreas and attributed the presence of enzymes in "secretin juice" to the mechanical washing out of granules from the cells. One gets the impression from recent publications from Dr. Babkin's laboratory that he no longer regards these experiments as conclusive. Barrington (1941) thought that in his experiments there was considerable change in the histological appearance of the cells following prolonged stimulation and Homans years ago, (1912) reported complete exhaustion of the pancreas with the disappearance of all granules from the cells when "secretin" was administered until either the pancreas failed to respond or the animal died. The problem is urgently in need of further study. Homans' experiments may be disregarded because the secretin available in 1912, particularly secretin of such a character that it would ultimately kill the experimental animal, might well cause changes which could not properly be attributed to secretin. Barrington, who used crystalline secretin, was himself unwilling to draw conclusions from the histological changes which he observed.

Hammarsten, Agren and Lagerlöf (1937) and Diamond, Siegel, Gall and Carlen (1939) report that secretin ("Pancreotest") increases the concentration of enzymes in the pancreatic juice in human subjects. A high degree of caution is advisable in accepting the results of such experiments inasmuch as it is probably impossible to obtain pure pancreatic juice by the clinical methods at present available. Voegtlin, Greengard and Ivy (1934) found that while secretin increased the total output of enzymes from the pancreas in both dogs and humans because of the increased volume of secretion, it almost invariably decreased the concentration.

The studies of Langstroth, McRae and Komarov (1939) offer a new approach to the problem. Using mathematical equations based on the assumption that secretin stimulates the production of protein material (enzymes) by the pancreas, these authors were able to predict with a high degree of accuracy the protein content of the pancreatic juice obtained by secretin stimulation under a variety of conditions. Since these assumptions also involve the disappearance of stored zymogen material from the cells during a relatively short period of activity, it should be possible to test the validity of their approach by means of histological studies. In the meantime their analysis must be regarded as providing strong support for the view that secretin stimulates, to some extent at least, the production of enzymes by the pancreas.

SUMMARY. It is evident that the secretion of enzymes by the salivary glands, the peptic glands of the stomach and by the pancreas is to a very large degree controlled through the nervous system and conversely it may be said that one of the most important functions of the nerves supplying the digestive glands is to regulate the secretion of enzymes. In the case of the salivary glands the secretion of water and salts is also entirely under nervous control. It is equally clear that in those glands which are stimulated through hormonal mechanisms the specific hormones activate chiefly those glandular elements responsible for the secretion of water, inorganic salts, acids or bases as the case may be. On the other hand, truly "secretory" influences in the Heidenhain sense are transmitted to the glands through secretory nerves. This is particularly the case with the salivary and gastric glands but is also true, though to a less extent, for the pancreas. Any conclusion regarding the possible "trophic" effect of the hormones would be decidedly premature.

REFERENCES

ALLEY, A. Am. J. Digest. Dis. and Nutr. **1:** 787, 1935. ANREP, G. V. J. Physiol. **49:** 1, 1914. ANREP, G. V. AND H. N. KHAN. J. Physiol. **58:** 302, 1924. ANREP, G. V., J. L. LUSH AND M. G. PALMER. J. Physiol. **59:** 434, 1925. BABKIN, B. P. Die äussere Sekretion der Verdauungsdrüsen. Berlin, 1914. Handbuch der normalen und pathologischen Physiologie, Berlin **3(BII):** 689, 1926. Am. J. Digest. Dis. and Nutr. **1:** 715, 1934a. Nature **134:** 1005, 1934b. The physiology of the salivary glands. In Dental Science and Dental Art, Philadelphia, p. 219, 1938a. Am. J. Digest. Dis. and Nutr. **8:** 467, 1938b. Personal communication to Dr. M. H. F. Friedman, 1942. BABKIN, B. P., A. ALLEY AND G. W. STAVRAKY. Trans. Roy. Soc. Canada **26(V):** 89, 1932. BABKIN, B. P., C. O. HEBB AND M. A. SERGEYEVA. Quart. J. Exper. Physiol. **29:** 217, 1939. BABKIN, B. P., W. J. RUBASCHKIN AND W. W. SAVITSCH. Arch. f. mikr. Anat. **74:** 68, 1909. BABKIN, B. P. AND W. W. SAVITSCH. Ztschr. f. physiol. Chem. **56:** 336, 1908; Centralbl. f. d. ges. Physiol. u. Pathol. des Stoffwechsels **4:** 225, 1909. J. russe de physiol. **3:** 143, 1921. Citation from Babkin,

1926. Babkin, B. P. and N. P. Tichomirov. Ztschr. f. physiol. Chem. **62:** 468, 1909. Barrington, E. J. W. J. Physiol. **100:** 80, 1941. Baxter, S. G. Am. J. Physiol. **96:** 349, 1931. Proc. Soc. Exper. Biol. Med. **29:** 511, 1932. Am. J. Digest. Dis. and Nutr. **1:** 36, 40, 1934. Blakely, A. P. L. and J. F. Wilkinson. Brit. J. Exper. Pathol. **14:** 349, 1933. Bloomfield, A. L. Ann. Intern. Med. **6:** 307, 1932. Bowie, D. J. Anat. Rec. **64:** 357, 1936; Bowie, D. J. and A. M. Vineberg. Quart. J. Exper. Physiol. **25:** 247, 1935. Brjuchanow, P. P. Ztschr. f. physiol. Chem. **87:** 364, 1913. Bucher, G. R., A. C. Ivy and J. S. Gray. Am. J. Physiol. **132:** 698, 1941. Bucher, G. R. and A. C. Ivy. Am. J. Physiol. **132:** 654, 1941. Bylina, A. Z. Arch. Sci. Biol. Inst. Méd. Expér. St. Petersburg **17:** 139, 1913. Crider, J. O. and J. E. Thomas. Proc. Philadelphia Physiol. Soc. Nov. 1941 (printed in Am. J. Med. Sci.). Crittenden, P. J. and A. C. Ivy. Am. J. Physiol. **119:** 724, 1937. Diamond, J. S., S. A. Siegel, M. B. Gall and S. Karlen. Am. J. Digest. Dis. **6:** 366, 1939. Edkins, J. S. J. Physiol. **34:** 133, 1906. Fleig, C. C. R. Soc. Biol. **55:** 462, 1903a. Centralbl. f. Physiol. **16:** 681, 1903b. Florovsky, G. Bull. de l'acd. des sciences, Petrograd 1917, p. 119. Citation from Babkin, 1926. Friedman, M. H. F. J. Cell. and Comp. Physiol. **5:** 83, 1934; **13:** 219, 1939. Frouin, A. C. R. Soc. Biol. **63:** 519, 1907. Gilman, A. and G. R. Cowgill. Am. J. Physiol. **97:** 124, 1931. Gray, J. S., M. S. Kim and A. C. Ivy. Am. J. Physiol. **116:** 210, 1936. Hammarsten, E., G. Agren and H. Lagerlöf. Acta med. Scand. **92:** 256, 1937. Citation from Harper and Vass, 1941. Harper, A. A. and C. C. N. Vass. J. Physiol. **99:** 415, 1941. Heidenhain, R. Studien d. physiol. Institut. zu Breslau **4:** 1, 1868. Citation from Babkin, 1914 and 1938. Pflüger's Arch. **10:** 599, 1875a; **10:** 557, 1875b; **17:** 1, 1878. Helmer, O. M., P. J. Fouts and L. G. Zerfas. J. Clin. Investigation **11:** 1129, 1932. Heymann, N. M. Diss. St. Petersburg, 1898. Citation from Babkin, 1914. Homans, J. Proc. Roy. Soc. London **86(3B):** 73, 1912. Ishikawa, T. Jap. J. Med. Sci. (I Anatomy), **2:** 22, 1929. Citation from Plenk, Handb. d. mikr. Anat. d. Mensch. **5**(Teil 2): 197, Berlin, 1932. Ivy, A. C. and A. J. Javois. Am. J. Physiol. **71:** 583, 1925. Klein, E. Arch. Surg. **26:** 246, 1933. Komarov, S. A. Proc. Soc. Exper. Biol. Med. **38:** 514, 1938. Revue Canad. de Biol. **1:** 191, 1942. Korovitsky, L. K. J. Physiol. **57:** 215, 1923. Kudrewetsky, B. B. Arch. f. Anat. u. Physiol. (Physiol. Abt.) 1894, s83. Kühne, W. and A. S. Lea. Untersuch d. physiol. Institut. zu Heidelberg, Bd. 2, 1882. Lalou, S. C. R. Acad. d. Sci. **151:** 824, 1910. Langstroth, G. O., D. R. McRae and S. A. Komarov. Canad. J. Research **17:** 137, 1939. Langley, J. N. J. Physiol. **2:** 261, 1879. Langley, J. N. and H. Sewall. Proc. Roy. Soc. London **29:** 383, 1879. Lim, R. K. S. and W. C. Ma. Quart. J. Exper. Physiol. **16:** 86, 1926. Meinberg, L. J. and C. L. Brown. Ann. Int. Med. **7:** 762, 1933. Mellanby, J. J. Physiol. **60:** 85, 1925. Metzner, R. Nagel's Handb. d. Physiol. d. Mensch. **2:** 899, Berlin, 1907. Morel, L. and E. Terroine. C. R. Acad. d. Sci. **67:** 36, 1909. Ostrogorski, S. A. Diss. St. Petersburg, 1894. Citation from Babkin, 1926. Oushakov, V. G. Diss. St. Petersburg, 1896. Citation from Vineberg, Am. J. Physiol. **96:** 363, 1931. Pavlov, I. P. Centralbl. f. Physiol. **2:** 137, 138, 1888; Arch. f. Anat. u. Physiol. (Physiol. Abt.) 1893 s. 176; Ergebn. d. Physiol. **3**(Abt. 1): 177, 1903; Nagel's Handb. d, Physiol. d. Mensch. **2:** 666, Berlin, 1907. Pavlov. I. P. and E. O. Schumowa Simanowskaja. Arch. f. Anat. u. Physiol. (Physiol. Abt.) 1895, s53. Polland, W. S. J. Clin. Investigation **11:** 449, 1932. Polland, W. S. and A. L. Bloomfield. J. Clin. Investigation **7:** 57, 1929. Popielski, L. Pflüger's Arch. **174:** 152, 1919. Pratt, C. L. G. J. Physiol. **98:** 1P, 1940. Rasenkov, J. P. Ztschr. f. exper. Med. (Russ.) **2:** 82, 1929. Citation from McKay and Baxter. Am. J. Physiol. **98:** 42, 1931. Rawlinson, H. E. Anat. Rec. **57:** 289, 1933; **63:** 295, 1935a; J. Anat. **70:** 143, 1935b Quart.; J. Exper. Physiol. **26:** 79, 1936. Rothlin, E. and R. Gundlach. Arch. intern. de Physiol. **17:** 59, 1921. Sacks, J., A. C. Ivy, J. P. Burgess and J. E. Vandolah. Am. J. Physiol. **101:** 331, 1932. Sacks, J. and M. S. Kim. Proc. Soc. Exper. Biol. Med. **27:** 182, 1929. Sanozki. Diss. St. Petersburg, 1893. Citation from Babkin, 1926. Savitsch, W. W. Centralbl. f. d. ges. Physiol. u. Pathol. des Stoffwechsels. **4:** 1, 1909. Scaffidi, V. Arch. f. Anat. u. Physiol. (Physiol. Abt.) 1907, s276. Sellheim, A. P. Diss. St. Petersburg, 1898. Citation from Babkin, 1914. Sergeyeva, M. A. Anat. Rec. **71:** 319, 1938. Stormont, D. L. Nerve endings and secretory activity in the submaxillary gland of the rabbit. Univ. of Chicago Published Theses, 1925. Citation from Stormont, 1932. Cowdry's Special cytology **1:** 153, New York, 1932. Thomas, J. E. and J. O. Crider. Am. J. Physiol. **134:** 656, 1941. Toby, G. C. Quart. J. Exper. Physiol. **26:** 45, 1936. Am. J. Digest. Dis. and Nutrition **3:** 896, 902, 1937. Tomaszewski, Z. Pflüger's Arch. **171:** 1, 1918. Vineberg, A. M. and B. P. Babkin. Am. J. Physiol. **97:** 69, 1931. Voegtlin, W. L., H. Greengard and A. C. Ivy. Am. J. Physiol. **110:** 198, 1934. Volborth, G. W. and N. N. Kudryavzeff. Am. J. Physiol. **81:** 154, 1927. Wertheimer, E. and LePage. J. de Physiol. et de Pathol. Gen. **3:** 693, 708, 1901. Wulfson. Diss. St. Petersburg, 1898. Citation from Babkin, 1914. Zeljony, G. P. and W. W. Savitsch. Verhandl. d. ges. russ. Arzte zu St. Petersburg, 1911–1912. Citation from Babkin, 1926.

Vol. 1, No. 3 **FEDERATION PROCEEDINGS** September, 1942

American Society of Biological Chemists

Symposium on Protein Metabolism

April 3, 1942

THE AMINO ACID REQUIREMENTS AND PROTEIN METABOLISM OF THE AVIAN ORGANISM

H. J. ALMQUIST

Division of Poultry Husbandry, University of California, College of Agriculture

Critical studies of the amino acid requirements of the chick have only recently become feasible. For some time it has been possible to maintain and grow rats on highly purified diets, while the more complicated nutritive requirements of the chick could not be met except by the use of natural feedstuffs. The chick is more sensitive to the lack of a number of dietary factors, including vitamin K, choline, manganese, potassium, magnesium, an essential carbohydrate, and certain unidentified substances in yeast. With the recognition and the ultimate provision of these factors in protein-free forms, it has become possible to conduct experiments on the amino acid requirements of chicks using diets in which the protein sources can be considered fairly well characterized.

Glycine. Glycine has been classified as nonessential, or dispensable, for the rat. A dietary complement of glycine is, however, necessary for the optimum growth of the young chick (1, 2, 3). This requirement amounts to approximately 1 per cent of the diet (1, 2, 4).

The chick probably does not lack the ability to synthesize glycine, but it is apparently unable to do so at a rate commensurate with the demands for early rapid growth. This is indicated by the data in table 1. The basal diet contained less than 0.1 per cent glycine or less than $\frac{1}{15}$ the optimal level, yet the basal group chicks were able to grow at $\frac{3}{4}$ of the optimal rate. It may be recalled that while other species detoxify benzoic acid by conjugation with glycine, the fowl utilizes ornithine for this purpose. This peculiarity of the fowl may be related to a difficulty in synthesizing or mobilizing glycine.

Chicks, apparently, have the ability to utilize acetates, which may serve as glycine substitutes in the diet (2). Glycollic acid and betaine are not effective as substitutes for glycine (2). Levels of 2 per cent or more of glycine added to the diet may be somewhat toxic to chicks (1) and heavy daily doses of 4 grams or more have proven fatal to hens (5).

Chicken embryos with chondrodystrophy contain less glycine than normal embryos (6), probably as a result of poorer formation of the cartilaginous and bony tissues, the proteins of which are especially rich in glycine. Some evidence has also been obtained of glycine synthesis in the developing chick embryo (6).

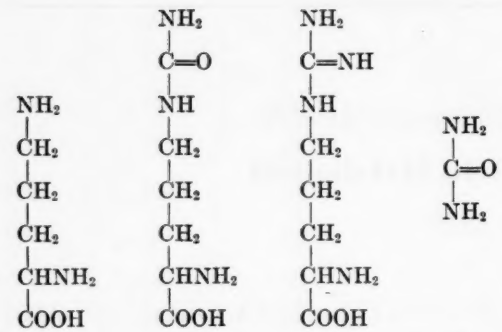
The glycine deficiency syndrome in the chick includes poor growth, muscular underdevelopment, a profound weakness, and a low muscle creatine content. It is particularly significant that this syndrome is preventable by feeding creatine (2, 4, 7, 8) for which glycine is evidently a precursor in the chick, as well as in the rat. The weakness has been identified with a dietary condition formerly thought to be a vitamin deficiency in the chick (7). Arginine also plays a prominent part in the development and prevention of the syndrome (7).

Coincident with the poor growth is an imperfect feather formation (9) which may be alleviated by adding creatine or sources of glycine and arginine to the diet (7, 8). Since chicken feathers contain 6 per cent arginine and 9.5 per cent glycine (10), it has been suggested that the observed greater effect of glycine and arginine deficiency in the case of a more rapidly feathering breed of chickens was due to the correspondingly greater demand for these amino acids for feather formation (7) as compared to a slowly feathering breed.

Young turkeys show no signs of a deficiency of glycine when fed a diet which will produce such a deficiency in chicks (9, 11).

Arginine. Unlike the rat, the young chick appears to lack any ability to synthesize arginine, and rapidly loses weight on a diet which is very low in arginine (12) (fig. 1). The arginine requirement of the young chick is high (13), probably

B & Z Library



1 per cent or more of the diet (12). While ornithine is unable to replace arginine, citrulline

TABLE 1

Growth-promoting effects of glycine in 30 per cent casein chick diets

SUPPLEMENT	LEVEL *per cent*	PERCENTAGE GAIN IN WEIGHT PER CHICK PER DAY	
		Expt. 1	Expt. 2
None		4.9	5.2
Glycine	0.5	5.2	
	1.0	5.9	6.3
	1.5	6.6	6.8
	2.0		5.9
Glycylglycine	1.0		6.8

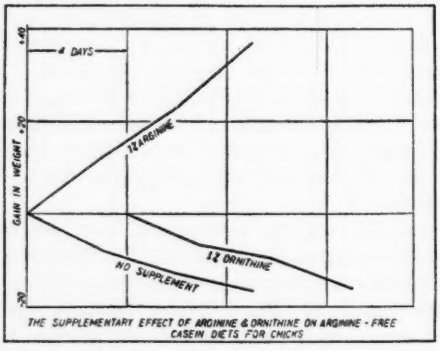

4 DAYS
GAIN IN WEIGHT
1% ARGININE
1% ORNITHINE
NO SUPPLEMENT
THE SUPPLEMENTARY EFFECT OF ARGININE & ORNITHINE ON ARGININE - FREE CASEIN DIETS FOR CHICKS

FIG. 1

is an effective substitute. This fact would indicate that the chick can replace the ureido oxygen of citrulline with an imino group (14) (fig. 2), or that it has a requirement for citrulline as such.

Since there appears no path from ornithine to citrulline, or to arginine in the chick, it is obvious that the Krebs-Henseleit urea cycle could not be completed. This seems consistent with the fact that the principal end-product of protein metabolism in birds is not urea but uric acid. The limited secretion of urea by birds may be accounted for by the small quantity of arginase found principally in the avian kidney. Chickens on a non-protein diet or on a diet containing an arginine-free protein hydrolysate excrete less than half the normal amounts of urea. When arginine or whole protein is given to such chickens, the urea excretion rises to a normal value (15). The urea formed by the chick embryo is not derived from the ammonia of general protein catabolism, but may be derived entirely from an active arginine-arginase system present at all stages from the second day of incubation on (16).

Methionine, cystine, homocystine and choline.— The essential nature of methionine for the chick has been clearly shown (17). Arachin, the principal protein of the peanut, is deficient in methionine, although well supplied with cystine. As the supplementary protein in chick diets, arachin will not support growth unless methionine and several other amino acids are added. It appeared necessary to add at least 0.75 per cent of dl-methi-

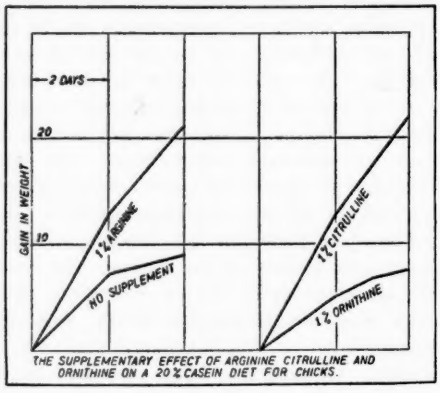
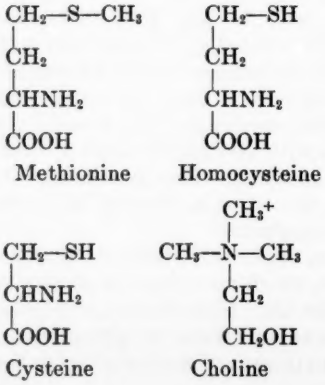


FIG. 2

onine to a chick diet containing 20 per cent arachin, 5 per cent gelatin and 5 per cent dried yeast to achieve an optimum rate of growth. On the other hand, cystine additions to the arachin diet did not promote chick growth (17). Approximately 0.5 per cent methionine in the diet may



be adequate for the chick in the presence of 0.4 per cent cystine. In the absence of cystine, approximately 0.9 per cent methionine may be required.[1] In the case of the rat, the corresponding requirement is 0.6 per cent methionine (17a).

Proteins of the raw soybean are not well utilized by rats or by chicks. It is well-known that the soybean protein is greatly improved by heat treatment. While additions of cystine to the raw soybean will correct part of the protein deficiency, the addition of methionine is much more effective (18). These results with the chick are in agreement with the conception that methionine may serve the purposes of both methionine and cystine, while cystine can meet only the requirements for cystine, but in so doing may exert a "sparing" action on methionine.

Homocystine will replace methionine efficiently in the chick only if ample choline is present in the diet (17) (fig. 3). In respect to the necessity

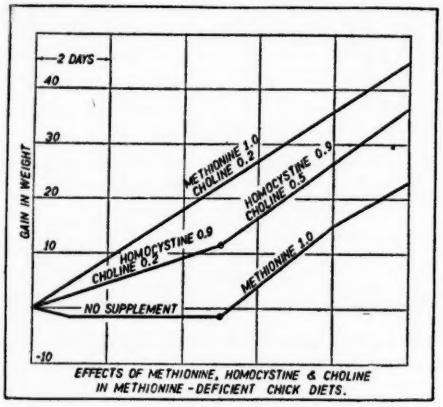


FIG. 3

of choline for the utilization of homocystine, the chick is completely analogous to the rat. It is noteworthy, however, that the level of choline required to insure efficient conversion of homocystine is several times that needed for other purposes.

While it has been indicated that methionine may exert some of the functions of choline in the rat, no such replacement is evident in respect to the perosis-preventing and growth-promoting effects of choline in the diets of chicks and turkeys (8, 19).

It has been shown that betaine is converted by rats into glycine by demethylation (20). It is also known that betaine can replace choline in the *diet* of rats as a source of methyl groups in the formation of methionine from homocysteine (21), and for other purposes (22). The chick, on the other hand, appears unable to utilize betaine to an appreciable extent as a precursor of glycine (2) or of choline (11, 23). Betaine did not improve the utilization of homocystine by chicks on a methionine deficient diet.[2]

Creatine formation in the chick. Three amino acids, glycine, arginine and methionine, are known to be involved in the biological synthesis of creatine in the rat. In the chick, the rôle of glycine and arginine in this synthesis has been demonstrated (2, 3, 4, 7) in terms of growth, tissue creatine content, and prevention of muscular weakness. Some typical results are given in table 2. It seems probable that methionine is also involved in the formation of creatine in the chick, as it is in the rat, but such action remains to be proven. In fact, a methionine-deficient diet which will not support growth does not appreciably inhibit the formation of muscle creatine in the chick. Furthermore, creatine, which exerts

TABLE 2

The effects of creatine and its precursors in the diet on the muscle creatine content of the chick

SUPPLEMENT	LEVEL	CREATINE CONTENT OF BREAST MUSCLE
	per cent	*mgm. per gm.*
None		3.48
Glycine	1.0	3.97
Glycine	1.0	4.42
Arginine	1.0	
Gelatin	10.0	4.53
Glycocyamine	1.2	5.00
Creatine hydrate	1.5	4.83
Creatinine	1.2	5.19

(Values corrected for non-creatine chromogens.)

a demonstrable sparing action in glycine and arginine deficient diets, seems to have no comparable effect in the case of methionine deficiency in chicks (17). Since methionine, which has contributed methyl groups in the synthesis of creatine, may conceivably be rapidly re-methylated by choline, only very small amounts of methionine from the diet or from the tissues may suffice for normal creatine synthesis *in vivo*.

In the following scheme, the biological formation of creatine in the chick is indicated, in so far as present evidence permits. For example; the improved growth and muscle creatine content on feeding glycocyamine (4) is undoubtedly due to the position of this compound as an intermediate stage in creatine formation in the chick, as well as in the rat, and to a sparing-action of glycocyamine on its immediate precursors, glycine and arginine. The similarly favorable effects that

[1] Unpublished data of the author.

[2] Unpublished data of the author.

are observed on feeding creatinine (4) are most simply explained by assuming that the reaction which forms creatinine from creatine is appreciably reversible in the chick. It should be added that glycocyamine and creatinine are not quite as effective as creatine in promoting growth, although all three elevate muscle creatine content equally well.

$$\text{Glycine} + \text{Arginine} \longrightarrow \text{Glycocyamine} \quad (+ \text{Orthinine?})$$

$$\text{Glycocyamine} + \text{Methionine} \longrightarrow \text{Creatine} \quad (+ \text{Homocysteine?})$$

$$\text{Creatine} \rightleftharpoons \text{Creatinine}$$

If ornithine is a by-product of the first step, it is evident that the arginine consumed cannot be regenerated in the chick (14). Assuming that homocysteine is a by-product of the second step, the regeneration of the methionine by reaction of homocysteine with choline (17) may be considered possible in the chick.

Tryptophane. The requirement of the young chick for tryptophane, as determined with a diet

TABLE 3

The growth-promoting effects of tryptophane in acid-hydrolysed casein diets for chicks

TRYPTOPHANE	PERCENTAGE GAIN IN WEIGHT PER CHICK PER DAY
per cent	
0.0	−2.3
0.2	+3.5
0.4	+4.4
0.5	+5.1
0.6	+5.2

in which the sole sources of protein were gelatin and acid-hydrolysed casein, is 0.44 to 0.5 per cent of the diet (24) (table 3). The optimum growth rate was not quite as high as that obtained when the hydrolysed casein plus tryptophane was replaced with whole casein. This has been a repeated result in amino acid studies with chicks; the most complete hydrolysates employed—even those prepared by the use of enzymes—having failed to support quite as rapid growth as the unhydrolysed proteins (12, 25) (fig. 4). The explanation for this finding is not apparent, and there seems to be no similar tendency shown in amino acid studies with rats. In view of the inferior results with hydrolysates, it would appear advisable to conduct investigations of the amino acid requirements of chicks as far as possible by the use of whole proteins.

Lysine. In a continuation of studies on the quantitative amino acid requirements of the young chick for optimum rate of growth (26), evidence was obtained on the level of lysine needed in the diet. The initial experiment was conducted with zein, since this protein contains no appreciable amount of lysine. After some failures in preliminary trials, the manifold deficiencies of zein were corrected by addition of pure amino acids, and satisfactory rates of growth of chicks were obtained.

Because of the necessity of many pure amino acid supplements to the zein diet and the corresponding high cost of this diet, experiments were attempted with edestin, a protein which appeared complete for the chick except for a partial deficiency of lysine and possibly methionine. Edestin is known to be a good source of

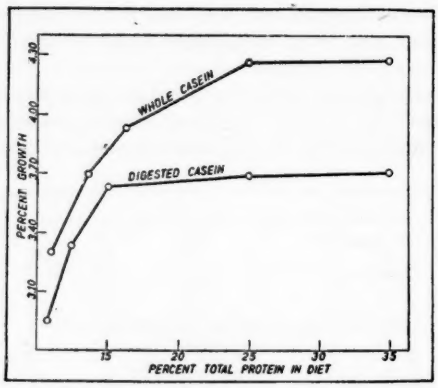


FIG. 4

TABLE 4

Growth-promoting effects of lysine additions to the edestin chick diet

TOTAL *l* (+)-LYSINE IN DIET	GAIN PER DAY
per cent	*per cent*
0.55	+3.3
0.65	+4.7
0.75	+5.9
0.85	+6.7
0.95	+6.8

threonine (27), concerning the distribution of which few quantitative data are yet available.

Diets were prepared as before, using as sources of amino acids edestin 25, glycine 1.0, and dl-methionine 0.4 per cent. The calculated lysine content of this diet was 0.55 per cent which was expected to be inadequate for optimum growth of the young chick, and so proved. *l*(+)-lysine monohydrochloride was added to the diet in amounts to adjust the total lysine content to the levels indicated in table 4. The results of this experiment and of the others indicate that a level of approximately 0.9 per cent lysine was needed for the highest rate of growth.

SUMMARY. 1. By the use of a highly purified diet which is essentially free of all sources of pro-

tein it is possible to study the amino acid requirements of the chick quantitatively.

2. Chicks have exhibited normal growth and development during the experimental intervals when the purified basal diet was supplemented with various well known proteins plus any necessary amino acid additions.

3. In respect to the requirement for glycine and arginine in the diet, the chick differs distinctly from the rat. These amino acids, however, play the same rôle in creatine formation in both species.

4. The interrelation of cystine and methionine and of homocystine, methionine and choline is the same in the chick as in the rat.

5. Tryptophane, lysine and histidine are essential for growth in the diet of the chick. The status of other amino acids for the chick remains to be investigated.

REFERENCES

(1) Almquist, H. J., E. L. R. Stokstad, E. Mecci and P. D. V. Manning. J. Biol. Chem. **134:** 213, 1940. (2) Almquist, H. J. and E. Mecchi. J. Biol. Chem. **135:** 355, 1940. (3) Hegsted, D. M., S. W. Hier, C. A. Elvehjem and E. B. Hart. J. Biol. Chem. **139:** 863, 1941. (4) Almquist, H. J., E. Mecchi and F. H. Kratzer. J. Biol. Chem. **141:** 365, 1941. Almquist, H. J. and E. Mecchi. Proc. Soc. Exper. Biol. Med. (in press). (5) Patton, A. R. Poultry Sci. **18:** 31, 1939. (6) Patton, A. R. and L. S. Palmer. J. Nutrition **11:** 129, 1936; Patton, A. R. J. Nutrition **13:** 123, 1937. (7) Hegsted, D. M., G. M. Briggs, C. A. Elvehjem and E. B. Hart. J. Biol. Chem. **140:** 191, 1941. (8) Jukes, T. H. J. Nutrition **22:** 315, 1941. (9) Jukes, T. H. Proc. Soc. Exper. Biol. and Med. **46:** 155, 1941. (10) Block, R. J. J. Biol. Chem. **128:** 181, 1939. (11) Jukes, T. H. J. Nutrition **20:** 445, 1940. (12) Klose, A. A., E. L. R. Stokstad and H. J. Almquist. J. Biol. Chem. **123:** 691, 1938. (13) Arnold, A., O. L. Kline, C. A. Elvehjem and E. B. Hart. J. Biol. Chem. **116:** 699, 1936. (14) Klose, A. A. and H. J. Almquist. J. Biol. Chem. **135:** 153, 1940. (15) Clementi, A. Arch. Sci. Biol. (Naples), **24:** 357, 1939. (16) Needham, J., J. Brachety and R. K. Brown. J. Exper. Biol. **12:** 321, 1935. (17) Klose, A. A. and H. J. Almquist. J. Biol. Chem. **138:** 467, 1941. (17a) Womack, M. and W. C. Rose. J. Biol. Chem. **141:** 375, 1941. (18) Hayward, J. W. and F. H. Hafner. Poultry Sci. **20:** 139, 1941. (19) Jukes, T. H. Poultry Sci. **20:** 251, 1941. (20) Stetten, D., Jr. J. Biol. Chem. **140:** 143, 1941. (21) Chandler, J. P. and V. du Vigneaud. J. Biol. Chem. **185:** 223, 1940. (22) Best, C. H. and M. E. Huntsman. J. Physiol. **75:** 405, 1932; Griffith, W. H. and D. J. Mulford. J. Am. Chem. Soc. **63:** 929, 1941. (23) Jukes, T. H. J. Nutrition **21:** 13, 1941. (24) Almquist, H. J. and E. Mecchi. Proc. Soc. Exper. Biol. and Med. **48:** 526, 1941. (25) Stokstad, E. L. R. Poultry Sci. **19:** 42, 1940. (26) Almquist, H. J. and E. Mecchi. Proc. Soc. Exper. Biol. and Med. **49:** 174, 1942. (27) Rose, W. C. Physiol. Rev. **18:** 109, 1938.

TRANSAMINATION

PHILIP P. COHEN

Department of Physiological Chemistry, University of Wisconsin, Madison

The demonstration that α-ketoglutaric and oxalacetic acids, the deamination products of glutamic and aspartic acids, are important, if not key intermediates in carbohydrate metabolism (1) has served to emphasize the intimate metabolic relationship of the carbohydrates and proteins. Pyruvic acid, the deamination product of alanine, had held this position for a long time because of its known importance as an intermediate metabolite of carbohydrates. Unfortunately, its link with protein metabolism through alanine did not permit any special emphasis since alanine has not been shown to possess any metabolic peculiarities which would entitle it to assume a special rôle. On the other hand, glutamic acid is known to be an intermediate in the metabolism of proline (2) and histidine (3), and possibly of citrulline (4) and lysine (45). In addition, glutamic and aspartic acids have been demonstrated to occupy a central position in amino acid and protein metabolism in the intact animal (5). The ready conversion of pyruvic acid to oxalacetic and α-ketoglutaric acids through the Krebs cycle and the carboxylation reaction has focused attention on these three compounds as representing the main intermediate links in the metabolism of proteins and carbohydrates.

The metabolic relationship of the amino acids alanine and aspartic acid with the corresponding α-keto acids has been demonstrated by the general reaction of oxidative deamination and reductive amination, and in the case of glutamic acid by the

specific enzyme, glutamic dehydrogenase (6, 7). Recently, a special mechanism has been discovered which is concerned with the intermolecular transfer of the amino group and a hydrogen atom from any one of these amino acids to the other two α-keto acids. This reaction has been termed transamination. Some properties of the transaminating enzyme system and its rôle in intermediary metabolism will be reviewed in this paper. (For more detailed reviews see 13 and 57.)

Nature of the reaction and its discovery. The interaction of α-amino and α-keto acids in the test tube has been known for a long time. Herbst (8) has more recently investigated this reaction in detail and has shown it to proceed as follows:

1) $RCH(NH_2)COOH + R_1COCOOH \rightarrow RCHO + R_1CH(NH_2)COOH + CO_2$

Reaction (1) takes place at high temperatures and is irreversible.

The biological analogue of Reaction (1) was first suggested by Needham (9) and later demonstrated by Braunstein and Kritzmann (10) to occur in pigeon breast muscle. Braunstein and Kritzmann showed that the reaction took place as follows:

2) $RCH(NH_2)COOH + R_1COCOOH \rightleftharpoons RCOCOOH + R_1CH(NH_2)COOH$

In contrast to Reaction (1), Reaction (2) is reversible, enzyme catalyzed, specific for l-amino acids, and yields no carbon dioxide. Since the intermolecular transfer of an amino group is involved in both reactions, the name transamination has been used in describing them. In this review the term transamination will be limited to the enzyme catalyzed, reversible system represented by Reaction 2.

METABOLITES ACTIVE IN TRANSAMINATION. *Amino and keto acids.* According to Braunstein and Kritzmann, (11, 19) the enzymatic transfer of amino groups in pigeon breast muscle takes place between any α-amino acid (with the possible exception of glycine) and the dicarboxylic acids, α-ketoglutaric and oxalacetic, as well as between the dicarboxylic amino acids, glutamic and aspartic, and various α-keto acids. No amino group transfer was observed in the following systems: 1, between monocarboxylic α-amino acids and monocarboxylic α-keto acids; 2, from amines and peptides; 3, from α-aminodicarboxylic acids to ketones, β-hydroxyketones or aldehydes.

In contrast to certain of the above findings the author (12) found that of 21 different α-amino acids studied in pigeon breast muscle l (−)-aspartic acid and l (+)-alanine were the most active in forming glutamic acid in the presence of α-ketoglutaric acid. α-aminobutyric acid and l (+)-valine showed a small activity, but none of the remaining amino acids was appreciably active. Of a series of α-keto acids studied, oxalacetic and pyruvic acids showed the greatest activity in causing the anaerobic disappearance of glutamic acid. α-ketobutyric and mesoxalic acids were slightly active, while no activity was observed with α-ketovaleric, α-ketocaproic, acetoacetic and laevulic acids. In addition, no transamination was observed between α-ketoglutaric acid and a variety of amino compounds other than α-amino acids. A highly specific analytical method for determining glutamic acid was employed (20).

d-Amino acids. Braunstein (13), Braunstein and Azarkh (14), and von Euler et al. (15) have reported slight transamination with d-amino acids. However, with the use of more accurate analytical methods the author was unable to demonstrate any significant transamination with different d-amino acids using both minced pigeon breast muscle (12) and purified transaminase preparations (16).

Peptides. The rôle of peptides as transamination substrates is of considerable interest. As noted above, Braunstein and Kritzmann (11) observed no activity with various peptides. In the case of transaminase preparations the author (16) was unable to demonstrate any appreciable transamination between glutathione and oxalacetic acid. On the other hand Ågren (17) reported that in minced cattle diaphragm muscle transamination takes place between α-ketoglutaric acid and the peptides glycylaminobenzoic acid and valylglycine. Valylglycine was found to be as active as alanine (about 30 per cent transamination), while glycylaminobenzoic acid was less active. Ågren employed the same method as Braunstein and Kritzmann for measuring transamination. Zorn (58) has recently shown this method to be unreliable for studying transamination. An accurate study of the rôle of peptides in transamination is obviously needed. It is possible that ketopeptides or peptides containing only certain combinations of amino acids are able to participate in this reaction.

PREPARATION AND PROPERTIES OF TRANSAMINATING ENZYMES. Kritzmann (22, 23, 24) has described in some detail the preparation and properties of purified transaminating enzymes from pigeon breast and pig heart muscles. According to her, there exist two distinct systems, one of which is concerned with glutamic acid (and α-ketoglutaric acid) and the other with aspartic acid (and oxalacetic acid). The former enzyme is called glutamic aminopherase and the latter aspartic aminopherase. Both enzymes are

reported to require co-factors, present in muscle kochsaft, of as yet unknown chemical constitution but which are similar, if not identical, for the two systems. Aspartic aminopherase is thought to be a more labile system since it is claimed that muscle suspensions lose their transaminating activity on dilution more rapidly with aspartic acid than with glutamic acid.

Transaminating enzyme preparations, termed transaminase, from pig heart and pigeon breast muscle have recently been studied by the author (16, 25). It was found that the activity of these preparations was greatest in the case Reaction 3.

3) l(+)-glutamic acid + oxalacetic acid $\underset{b}{\overset{a}{\rightleftharpoons}}$ α-ketoglutaric acid + l(−)-aspartic acid

That is, the enzyme was most active in catalyzing a reaction in which *both* glutamic and aspartic acids (and the corresponding α-keto acids) were substrates. The addition of pigeon breast muscle kochsaft was without influence on the rates of Reactions 3, 4, or 5.

4) l(+)-glutamic acid + pyruvic acid $\underset{b}{\overset{a}{\rightleftharpoons}}$ α-ketoglutaric acid + l(+)-alanine

5) l(−)-aspartic acid + pyruvic acid $\underset{b}{\overset{a}{\rightleftharpoons}}$ oxalacetic acid + l(+)-alanine

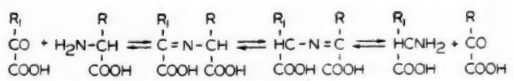
Reaction 3 was catalyzed at a rapid rate, the Qtransamination values being of the order of 1600. The position of equilibrium for this system was far to the right, with an equilibrium constant of about 3. Reaction 4 was catalyzed at a much slower rate, the Qtransamination values being of the order 300. The equilibrium constant was about 1. Reaction 5 was not catalyzed by transaminase.

Substrate specificity. Experiments with purified enzyme preparations, transaminase, showed essentially the same substrate specificity as with pigeon breast muscle (16). Thus, with α-ketoglutaric acid, only l(−)-aspartic acid, l(+)-alanine, and l(−)-cysteic acid were active, while with oxalacetic acid only l(+)-glutamic and l(−)-cysteic acids reacted. A few experiments have been reported by Braunstein (13) in which glutamic acid formation from α-ketoglutaric acid and different amino acids was measured with both pigeon breast muscle and purified enzyme preparations. Of interest are his findings that while l(+)-alanine shows a slightly smaller activity with the pigeon breast muscle, l(+)-valine, l(−)-leucine, and l(+)-isoleucine react to only about one-third the extent with purified enzyme. These differences, no doubt, reflect the inadequacy of the analytical method employed by these investigators. (See 58.)

From the studies with purified transaminase (16, 25) it appeared that the chief substrates for this enzyme are those represented in Reaction 3. Aside from pyruvic acid and l(+)-alanine, no mono-basic α-amino, or α-keto acids were found to be active. However, dibasic α-keto and α-amino acids other than those shown in Reaction 3 are active. Thus l(−)-cysteic acid will react with both oxalacetic and α-ketoglutaric acids.

Braunstein (13) and Bychkov (21) have reported that cysteic acid and phosphoserine are active in transamination in pigeon breast muscle. The activity of cysteic acid was confirmed using purified transaminase (16) but phosphoserine was found to be inactive. Of interest, however, is the report by Braunstein (13) that neither of these two compounds is active with glutamic and aspartic aminopherases.

MECHANISM OF TRANSAMINATION. The mechanism by which the intermolecular transfer of the amino group takes place is not known. Following Herbst's (8) idea of the mechanism of non-enzymatic transamination, Braunstein and Kritzmann (10) have postulated the formation of an intermediate Schiff's base, and picture the reaction as follows:

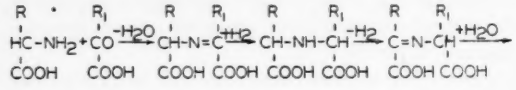


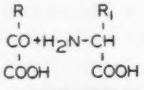
SCHEME I

The inability of purified transaminase preparations to dehydrogenate glutamic or aspartic acids in the presence of suitable hydrogen acceptors, and the absence of free NH_3 during the course of the reaction suggest that the amino group is transferred through an intermediate complex of the Schiff's base type.

Karrer et al. (27) have investigated the metabolism of octopin, following the suggestion of Knoop and Martius (28) that octopin represents a naturally occurring intermediate compound of transamination. Karrer et al. found that fresh liver brei, which they state contains an l-amino acid dehydrogenase, was capable of dehydrogenating octopin. d-amino acid dehydrogenase preparations were not active, indicating that the alanine was of the l-form. Since the natural synthesis of octopin involves an intermediate reduction of a Schiff's base to an α,α′-iminodicarboxylic acid, the authors conclude that transamination may

involve an intermediate reduction of the Schiff's base with subsequent dehydrogenation. The reaction is pictured as follows:





SCHEME II

As previously pointed out, the evidence for the existence of a cofactor for transaminase is still inconclusive. Should an oxidizable and reducible cofactor be shown to be involved, its function might be that of acting as the hydrogen acceptor and donator in the scheme of Karrer et al. (scheme II). However, as mentioned previously, none of the known cofactors have any influence on transaminase activity, notwithstanding the fact that the method of transaminase preparation is such as to remove practically all the known cofactors with the possible exception of flavine-adenine-dinucleotide.

In a later paper Karrer et al. (29) reported on the oxidation of racemic α,α'-iminodipropionic acid by d- and l-amino acid oxidase, the latter a liver brei preparation. The compound was found to be oxidized by both preparations. According to Karrer et al., the interconversion of d- and l-amino acids may take place biologically through an intermediate of this type.

TRANSAMINATION IN DIFFERENT TISSUES. Much of the available data on transamination is of a qualitative nature. In addition to demonstrating that transamination occurs it is essential to know the rate of the reaction in terms of unit weight of that tissue. Thus, a significant amount of transamination may be shown to take place in certain cases with large amounts of tissue and long incubation periods (18), but calculation of the rates in terms of Q transamination would reveal a value so low as to place doubt on the significance of this reaction in the metabolism of that tissue. In many tissues the rates of other reactions, both aerobic and anaerobic, involving the same metabolites may proceed at rates so much faster as to preclude the possibility of transamination taking place. It is therefore essential to have accurate data on the rate of transamination in different tissues before it can be assigned a rôle in intermediary metabolism. Unfortunately, very little such data is available. In table 1 are listed all the data available to the author on transamination in different tissues. The results of other workers have been recalculated and expressed as Qtransamination values.

$$Q\ \text{transamination} = \frac{\text{microliters substrate transaminated}}{\text{mgm. dry weight} \times \text{hr.}}$$

Animal tissues. As can be seen from table 1, the system represented by Reaction 3 is the most active in all the tissues studied. This reaction is so rapid in muscle tissue that in the presence of l(+)-glutamic acid oxalacetic acid will be preferentially transaminated, notwithstanding the rapid rates of other reactions involving this metabolite. Liver, kidney, and brain, while less active than muscle, also catalyze Reaction 3 at a relatively rapid rate. The Q values for Reaction 3a are in most instances greater than the Qsuccinoxidase and Qcytochrome oxidase values reported for the same tissues.

It is apparent from table 1 that the rates of Reactions 4 and 5 are comparatively very slow in all tissues studied, with the exception of pigeon breast muscle in the case of Reaction 4, and of liver in the case of Reaction 4a. As a matter of fact in most of these tissues the rates of Reactions 4 and 5 are so slow in comparison with other reactions involving the same metabolites, that it is highly doubtful that transamination involving these systems plays any significant metabolic rôle.

Embryonic and tumor tissue show considerably slower transamination rates than the corresponding adult or normal tissues.

Plant tissues. No accurate quantitative experiments on transamination in plant tissues have been published. However, qualitative experiments have been reported (7, 23, 35, 36, 37) and on the basis of these, elaborate theories of the rôle of transamination in plant metabolism have been advanced. The most complete study of this reaction in plants has been carried out by Cedrangolo and Carandante (38). Unfortunately, their study suffers from the use of poor analytical methods. From their experiments with different extracts of seeds it is apparent that the rates of transamination are very slow. However, it should be noted (table 1) that in all instances Reaction 3b is more rapid than 4b, and in most instances Reaction 4b is faster than 5a. This would seem to indicate that in plants reactions involving aspartic acid are more rapid than those involving glutamic acid. The existence of an aspartic aminopherase in plants has been reported by Kritzmann (23). However, it is obvious that as in the case of animal tissues, the fastest rates are realized when the substrate pair includes both a dibasic α-amino acid and a dibasic α-keto acid.

Chlorella shows no transaminase activity.

Bacteria and yeast. As can be seen from table 1, the transamination reaction is much slower in B. coli and brewer's yeast than in animal tissues. Baker's yeast appears to have no transaminase activity. Qualitative experiments on trans-

TABLE 1

Transamination for different animal and plant tissues

TISSUE	REACTION	QTRANSAMINATION	REFERENCES
Animal			
Muscle:			
Skeletal (rat)	3a	316	30
	3b	135	30
	4a	13	30
	4b	4	30
	5a	1	30
	5b	2	30
Skeletal (cat)	3a	20	31
Diaphragm (beef)	4a	3	17
	4b	3	17
Breast (pigeon)	3a	450	26
	3b	160	26
	4a	50	12
	4a	28	10
	4b	42	12
	4b	36	10
	5b	1	*13
Heart (rat)	3a	425	30
	3b	190	30
	4a	7	30
	4b	4	30
	5a	7	30
	5b	2	30
Heart (rabbit)	4a	1	32
Heart (dog)	5b	1	13
Liver (rat)	3a	245	30
	3b	102	30
	4a	46	30
	4b	3	30
	5a	10	30
	5b	3	30
Liver (cat)	3a	230	33
	3a	14	31
Liver (rabbit)	4a	1	32
	5b	1	13
Kidney (rat)	3a	245	30
	3b	112	30
	4a	3	30
	4b	3	30
	5a	3	30
	5b	2	30
Kidney (cat)	3a	220	33
	3a	23	31
Kidney (rabbit)	4a	1	32
	5b	1	13
Brain (rat)	3a	260	30
	3b	107	30
	4a	2	30
	4b	3	30
	5a	8	30
	5b	1	30
Brain (cat)	3a	220	33
	3a	20	31
Brain (rabbit)	4a	2	32
	5b	0.5	13
Lung (rat)	3a	51	30
Lung (cat)	3a	23	31
Testis (rat)	3a	150	30
Spleen (rat)	3a	16	30
Erythrocytes (cat)	3a	3	31
Defibrinated blood (human)	3a	0	26
Serum (human)	3a	0	26
Mammary gland, lactating (guinea pig)	4b	3	26

TABLE 1—*Continued*

TISSUE	REACTION	QTRANSAMINATION	REFERENCES
Animal—*Continued*			
Embryonic (cat):			
Liver	3a	64	33
Kidney	3a	78	33
Brain	3a	77	33
Muscle	3a	20	31
Placenta	3a	94	33
Tumors:			
No. 108 (mouse)	3a	72	33
	3b	3	33
	4a	0.4	33
	4b	0.6	33
Yale no. 1 (mouse)	3a	54	33
	3b	2	33
	4a	0.7	33
	4b	0.6	33
No. 15091-A (mouse)	3a	54	33
	3b	3	33
	4a	0.3	33
	4b	1.0	33
S-37 (mouse)	3a	57	33
	4a	0.7	33
No. 42 (mouse)	3a	72	33
	4a	0.7	33
U.S.P.H.S. No. 17 (mouse)	3a	53	33
	4a	0.5	33
Hepatoma (rat)	3a	64	34
Brown-Pearce carcinoma (rabbit)	4a	0–0.6*	14
	4b	0.9*	14
Jensen sarcoma (rat)	4a	0–0.8*	14
	4b	0.2*	14
Sinelnikov - Krichensky sarcoma (rat)	4a	0.4*	14
Rous sarcoma (chicken)	4a	0–0.2*	14
Fischer sarcoma (chicken)	4a	1 *	14
	4b	0.2*	14
Plants			
Species and preparations:			
Chlorella (cells)	3b	0	26
	4b	0	26
Seeds—aqueous extracts; acetone precipitated and dialyzed:			
Corn	3b	1.4	35
	4b	0.8	35
	5a	0.4	35
Pea	3b	0.9	35
	4b	0.7	35
	5a	0.4	35
Seeds—aqueous extracts; $(NH_4)_2SO_4$ precipitated and dialyzed:			
Corn	3b	1.5	35
	4b	.0	35
	5a	1.2	35
Barley	3b	4.4	35
	4b	0.3	35
	5a	2.2	35
Pea	3b	1.1	35
	4b	.0	35
	5a	0.4	35
Clover	3b	1.7	35
	5a	1.6	35

* Calculated on basis of experimental conditions used in other published experiments.

TABLE 1—*Concluded*

TISSUE	REACTION	QTRANSAMINATION	REFERENCES
Plants—*Continued*			
Species and Preparation—Con't.			
Germinated seedlings — aqueous extracts; $(NH_4)_2SO_4$ precipitated and dialyzed:			
Corn	3b	3.8	35
	4b	1.3	35
	5a	2.9	35
Pea	3b	1.1	35
	4b	1.0	35
	5a	0.7	35
Bacteria and yeast			
ORGANISM	REACTION	QTRANSAMINATION	REFERENCE
Brewer's yeast (aqueous extract)	3b	10	26
Baker's yeast	3b	0	26
	4b	0	26
B. coli	3a	17	26

amination with yeast extracts and B. coli have been reported by Adler et al. (35, 39).

ROLE OF TRANSAMINATION IN INTERMEDIARY METABOLISM. A direct method for determining the rôle of transamination in intermediary metabolism is suggested by the use of a specific inhibitor. Unfortunately, such a compound has not been discovered to date.

From the experimental evidence available, there is as yet no indication that transamination plays a single specific rôle in intermediary metabolism. The substrates of this reaction are highly reactive and participate in many different rapid metabolic processes. Obviously, if transamination is to play a significant rôle in cellular metabolism it must be shown to proceed at a rate rapid enough to be quantitatively significant. From Table I it is apparent that truly rapid rates are seen only with the system glutamic acid plus oxalacetic acid (Reaction 3). The rapid rates at which oxalacetic, α-ketoglutaric and pyruvic acids participate in non-transaminating reactions make it highly doubtful that reactions 4 and 5 ever proceed at rates fast enough to participate in the metabolism of these compounds. As previously stated, this may not apply to liver and pigeon breast muscle.

Among the possible metabolic reactions which transamination may influence are those of protein and amino acid synthesis and degradation, glycolysis, and hydrogen transport.

Protein Metabolism. Animal tissues. The central rôle which the dibasic α-amino acids, glutamic and aspartic, appear to occupy in protein metabolism has led to an emphasis of all metabolic reactions involving these compounds. Inasmuch as glutamic acid is the only amino acid which has a specific enzyme system concerned with its synthesis and oxidative deamination, Euler et al. (7) have suggested that the synthesis of glutamic acid from α-ketoglutaric acid and NH_3 is the first step in amino acid synthesis in animal tissues. On the basis of Braunstein's claim that glutamic acid will react with all α-keto acids by transamination, the synthesis of other amino acids would be expected to take place by means of this reaction. Conversely, α-ketoglutaric acid would be expected to react with any amino acid to yield glutamic acid plus the corresponding α-keto acid. Thus, the synthesis and degradation of practically all the amino acids could be explained on the basis of these two enzyme systems.

While kidney and liver are known to be capable of oxidizing most amino acids, other tissues, such as muscle and brain, are limited to the oxidation of glutamic and aspartic acids (9), and glutamic acid respectively (40, 41). The rôle of transamination in these tissues in particular would appear important as a mechanism for the utilization of other amino acids. However, the very fact that in vitro other amino acids are not utilized by these tissues presents a real objection to this theory, since both enzyme systems are known to occur in these tissues, though in varying amounts. As previously stated, even in the presence of equimolar concentrations of α-ketoglutaric acid the author found transamination to be limited to only a few amino acids of a large number tried with muscle tissue (12).

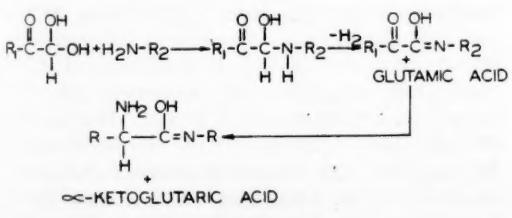
On the other hand, the possible rôle of these two enzyme systems in kidney and liver has been pointed out by Braunstein and Bychkov (42). Thus, they reported that NH_3 is produced from l(+)-alanine when the latter is incubated with α-ketoglutaric acid, glutamic aminopherase, glutamic dehydrogenase, pyocyanine and cozymase. About 12.5 per cent of the theoretical yield was realized after three hours incubation. According to these investigators, this coupled system represents a cell free model of l-amino acid dehydrogenase, the existence of which as an entity has not been demonstrated to date. The known facts about amino acid metabolism in these tissues, however, do not support this suggestion.

In the first place, the rate of deamination of the different amino acids is faster in kidney than in liver (43). Since liver contains more than twice as much glutamic dehydrogenase as kidney (7) and about the same amount of transaminase (see table 1), it would be expected that liver would be more active than kidney if these two systems were required for deamination. Secondly, the

rate of NH_3 production from aspartic acid is almost twice as fast as from glutamic acid in kidney cortex (36). This in itself would constitute strong evidence against the deamination system proposed by Braunstein and Bychkov. Of course the possibility exists that kidney contains a specific aspartic acid dehydrogenase which together with transaminase could account for the deamination of certain amino acids. However, there is no evidence that such an enzyme exists. Further, should such an enzyme be shown to exist, its chief rôle, aside from aspartic acid deamination, would be that of deaminating glutamic acid, due to the rapid rate at which the latter is converted to aspartic acid through transamination with oxalacetic acid. As can be seen from table 1, this is the fastest transamination reaction in all tissues studied. Thirdly, it should be pointed out that the rates of aerobic and anaerobic metabolism of α-ketoglutaric and oxalacetic acids in kidney and liver are far more rapid than the rates of transamination with monocarboxylic amino acids, the majority of which are not at all active in transamination. Consequently, the opportunity for utilizing these highly active metabolites in slow transamination reactions is extremely doubtful. Finally, experiments by the author (26) failed to demonstrate any appreciable increase in NH_3 production from various combinations of α-ketoglutaric acid and l-amino acids. Homogenized liver and kidney, fortified with cozymase and methylene blue were used.

Borsook and Dubnoff (45) have recently reported that glutamic and aspartic acids react with citrulline aerobically in the presence of kidney slices to form arginine. This reaction has been termed transimination. Expressed in terms of Qvalues, transimination proceeds at about the same rate as Reactions 4 and 5 with rat kidney tissue (table 1).

A possible metabolic relationship between transamination and protein synthesis in animal tissue has been pointed out by Linderstrøm-Lang (44). On the basis of the plausible assumption that the synthesis of protein occurs by a metabolic mechanism other than the reversal of proteolysis, Linderstrøm-Lang postulated the following scheme:

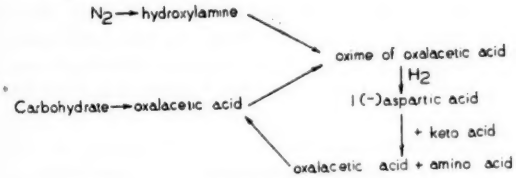


SCHEME III

As can be seen from scheme III, a keto-aldehyde reacts with an amine to form a ketonic Schiff's base. The latter then reacts with glutamic acid via the transamination reaction to yield a peptide. Ågren (17) has recently published unconvincing evidence in support of this.

On the basis of the above it would be expected that in tissues where rapid protein synthesis was taking place, e.g., embryonic and tumor tissue, the transaminase activity would be higher than in normal adult tissues. Actually, the reverse has been found to be the case (33). Thus, it was observed that in tumors and embryonic tissue the transaminase activity was low as compared with normal adult tissues. This apparent inverse relationship between protein synthesis and transamination suggests that the latter reaction may serve as a controlling mechanism in protein synthesis.

Plant tissues. The possible rôle of transamination in plant protein synthesis is suggested from the following scheme of Virtanen and Laine (46) for leguminous plants:



SCHEME IV

Some experimental evidence for scheme IV has been reported by Virtanen and Laine (36), and critically discussed by Wilson (37). It is possible that transamination plays an important rôle in the synthesis of amino acids in plants. However, until careful, quantitative studies are carried out, the rôle of this reaction in plant metabolism will continue to remain obscure.

Transamination and glycolysis. Krebs (47) and Weil-Malherbe (48) observed that glycolysis in retina and brain tissue was inhibited by glutamic acid. This effect has been studied in more detail recently by Grodzensky (49) who was able to show that anaerobic glycolysis in pigeon breast muscle was also inhibited (20–50 per cent) by glutamic acid. Further, he was able to demonstrate that the inhibition was due to conversion of pyruvic acid to alanine by transamination with glutamic acid.

The influence of glutamic acid on glycolysis in tumors was investigated by the author (26). Inhibitions of the order of 10 to 15 per cent were observed. These results are in keeping with the finding that tumors have a low transaminase content (33).

Transamination and hydrogen transport. Transamination is not only a very rapid reaction but it

is also concerned chiefly with these substances which are known to play key rôles in intermediary metabolism. Thus, oxalacetic, α-ketoglutaric, glutamic and aspartic acids all catalytically influence respiration (9, 50, 51, 52, 53). Further, glutamic acid has been found to function as a hydrogen carrier not only because its dehydrogenase can act with both di- and tri-phosphopyridine nucleotides (7, 54) and so can couple with other di- and tri-phosphopyridine nucleotide catalyzed systems (54, 55), but also because of its rôle in a dismutation reaction involving α-ketoglutaric acid and NH_3 (56).

Thus, the chief rôle of transamination may be that of rapidly interconverting certain of the respiratory mediators. That the transamination reaction is fast enough in the case of the dibasic α-keto and α-amino acids to successfully compete with other metabolic reaction involving the same substrates has been previously indicated.

In conclusion, it appears that transamination represents a "shuttle" mechanism whereby certain key protein and carbohydrate intermediates are rapidly interconverted. The fact that transamination is more or less limited to these key compounds does not minimize its importance, but rather emphasizes the need for further investigation of the intermediary metabolism of amino acids.

REFERENCES

(1) Krebs, H. A. and W. A. Johnson. Biochem J. **31:** 645, 1937. (2) Weil-Malherbe, H. and H. A. Krebs. Biochem. J. **29:** 2077, 1935. (3) Edlbacher, S. and M. Neber. Ztschr. physiol. Chem. **224:** 261, 1934. (4) Bach, S. J. Biochem. J. **33:** 1833, 1939. (5) Schoenheimer, R. and D. Rittenberg. Physiol. Rev. **20:** 218, 1940. (6) Dewan, J. G. Biochem. J. **32:** 1378, 1938. (7) von Euler, H., E. Adler, G. Günther and N. B. Das. Ztschr. physiol. Chem. **254:** 61, 1938. (8) Herbst, R. M. J. Am. Chem. Soc. **58:** 2239, 1936. (9) Needham, D. M. Biochem. J. **24:** 208, 1930. (10) Braunstein, A. E. and M. G. Kritzmann. Enzymologia **2:** 129, 1937. (11) Braunstein, A. E. and M. G. Kritzmann. Biochimia, U. S. S. R. **3:** 590, 1938. (12) Cohen, P. P. Biochem. J. **33:** 1478, 1939. (13) Braunstein, A. E. Enzymologia **7:** 25, 1939. (14) Braunstein, A. E. and R. M. Azarkh. Nature **144:** 669, 1939. (15) von Euler, H., H. Hellström, G. Günther, L. Elliott and G. Elliott. Ztschr. physiol. Chem. **259:** 201, 1939. (16) Cohen, P. P. J. Biol. Chem. **136:** 565, 1940. (17) Ågren, G. Acta Physiol. Scand. **1:** 233, 1940. (18) Cohen, P. P. Proc. Am. Soc. Biol. Chem., J. Biol. Chem. **133:** xx, 1940. (19) Braunstein, A. E. and M. G. Kritzmann. Biochimia, U. S. S. R. **4:** 168, 1939; Chem. Abstr. **34:** 1694, 1940. (20) Cohen, P. P. Biochem. J. **33:** 551, 1940. (21) Bychkov, S. M. Biochimia, U. S. S. R. **4:** 189, 1939; Chem. Abstr. **34:** 1694, 1940. (22) Kritzmann, M. G. Biochimia, U. S. S. R. **3:** 603, 1938. (23) Kritzmann, M. G. Nature **143:** 603, 1939. (24) Kritzmann, M. G. Biochimia, U. S. S. R. **4:** 667, 1939; Chem. Abstr. **34:** 5865, 1940. (25) Cohen, P. P. J. Biol. Chem. **136:** 585, 1940. (26) Cohen, P. P. Unpublished experiments. (27) Karrer, P., H. Koenig and R. Legler. Helv. Chim. Acta **24:** 127, 1941. (28) Knoop, F. and C. Martius. Ztschr. physiol. Chem. **254:** x, 1938. (29) Karrer, P., H. Koenig and R. Legler. Helv. Chim. Acta **24:** 861, 1941. (30) Cohen, P. P. and G. L. Hekhuis. J. Biol. Chem. **140:** 711, 1941. (31) Breusch, F. L. Biochem. J. **33:** 1757, 1939. (32) Kritzmann, M. G. Enzymologia **5:** 44, 1938. (33) Cohen, P. P. and G. L. Hekhuis. Cancer Res. **1:** 620, 1941. (34) Cohen, P. P., G. L. Hekhuis and E. K. Sober. Cancer Res. (in press). (35) Adler, E., G. Günther and J. E. Everett. Ztschr. physiol. Chem. **255:** 27, 1938. (36) Virtanen, A. I. and T. Laine. Biochem. J. **33:** 412, 1939. (37) Wilson, P. W. The biochemistry of symbiotic nitrogen fixation. Univ. Wisconsin Press, Madison, 1940. (38) Cedrangolo, F. and G. Carandante. Arch. di Scienze Biol. **26:** 369, 1940. (39) Adler, E., H. Hellström, G. Günther and H. von Euler. Ztschr. physiol. Chem. **255:** 14, 1938. (40) Krebs, H. A. Biochem. J. **29:** 1620, 1935. (41) Weil-Malherbe, H. Biochem. J. **30:** 665, 1936. (42) Braunstein, A. E. and S. M. Bychkov. Nature **144:** 751, 1939. (43) Krebs, H. A. Ztschr. physiol. Chem. **217:** 191, 1933. (44) Linderstrøm-Lang, K. Ann. Rev. Biochem. **7:** 37, 1939. (45) Borsook, H. and J. W. Dubnoff. J. Biol. Chem. **141: 717,** 1941. (46) Virtanen, A. I. and T. Laine. Nature **141:** 748, 1938. (47) Krebs, H. A. Biochem. J. **29:** 1951, 1935. (48) Weil-Malherbe, H. Biochem. J. **32:** 2257, 1938. (49) Grodzensky, D. E. Bull. biol. med. exper. U. S. S. R. **9:** 116 1940; Chem. Abstr. **35:** 2535, 1941. (50) Annau, E., I. Banga, A. Blazso, V. Bruckner, K. Laki, F. B. Straub and A. Szent-Györgyi. Ztschr. physiol. Chem. **245:** 118, 1937. (51) Krebs, H. A. Biochem. J. **34:** 775, 1940. (52) Krebs, H. A. and L. V. Eggleston. Biochem. J. **34:** 442, 1940. (53) Baumann, C. A. and F. J. Stare. J. Biol. Chem. **133:** 183, 1940. (54) Adler, E., H. von Euler, G. Günther and M. Plass. Biochem. J. **33:** 1028, 1939. (55) Dewan, J. G. Biochem. J. **33:** 549, 1939. (56) Krebs, H. A. and P. P. Cohen. Biochem. J. **33:** 1895, 1939. (57) Cohen, P. P. Symposium on respiration. Univ. Wisconsin Press (Madison, 1942). (58) Zorn, K. Ztschr. physiol. Chem. **266:** 239, 1940.

THE METABOLISM OF THE *d*-AMINO ACIDS[1]

CLARENCE P. BERG

Biochemical Laboratory, State University of Iowa, Iowa City

For some time it has been rather axiomatically assumed that natural products yield only *l*-amino acids on hydrolysis and that if *d*-amino acids are found, they must have been produced by racemization (2). In the last five years several studies have suggested that there may be exceptions to this general rule.

Isolations of *d*-proline from the alkaloids of ergot and of *d*(−)-glutamic acid from the capsular material of Bacillus anthracis and Bacillus mesentericus were reported in 1935 and 1937 (3).

In 1939 Kögl (4) announced that hydrolysates of the protein of malignant tumors were characterized by their high content of *d*-amino acids, sometimes present in proportions representing 5 per cent of all of the amino acids isolated. The optical rotation of the glutamic acid was low enough in several instances to suggest that 16 to 44 per cent existed in the *d* form. By contrast, amino acids similarly isolated from normal tissues showed only slightly low or normal rotation.

The few confirmations and the several denials of this assertion were concerned almost entirely with glutamic acid alone and have been too recently reviewed to warrant their consideration in detail. The accumulated evidence casts serious doubt on Kögl's original claim, but some of the fundamental technical considerations deserve emphasis because they illustrate difficulties inherent in studies pertaining to the optical isomerism of the amino acids. In most instances Kögl isolated only a small part of the total glutamic acid content of the tissue and apparently assumed that his sample was representative. Chibnall and his associates contend that the cuprous oxide method used by Kögl favors the isolation of *dl*-glutamic acid and hence yields erroneously high estimates of *d*(−)-glutamic acid when based on incomplete isolation (5). Kögl and Erxleben (6) argue that calcium *dl*-glutamate is much more soluble in aqueous ethyl alcohol solutions than calcium *l*-glutamate and that therefore the *d*-acid may be overlooked in the Foreman method used by Chibnall. On the contrary, Graff, Rittenberg, and Foster (7) could find no difference in the solubilities of the calcium and barium salts of the natural and the racemic forms with the isotopic dilution procedure of estimation. Tests with *d*-amino acid oxidase (8) showed no greater concentration of *d*-amino acids in hydrolysates of proteins from malignant tissue than in hydrolysates of normal tissue proteins in 20 hours, but Kögl, Herken and Erxleben (9) contend that errors due to extraneous reactions become so greatly magnified in such an extended period that careful differention is not possible. Applying this method for periods of 90 minutes to two glutamic acid preparations from malignant tumors, they found no significant difference in oxygen absorption and ammonia production even though calculations from polariscopic measurements had indicated that one sample contained 43, the other 30.5 per cent, of the *d* isomer. Behrens, Lipmann, Cohn, and Burk (10) observe that adsorbed impurities like inorganic chlorides, which do not readily affect the melting point of glutamic acid, and amino acids, which are not readily detected by elementary analysis, can be removed only by repeated recrystallizations; the presence of either of these may account for low responses to *d*-amino acid oxidase, coincident with low optical rotations. The controversy has at least served to direct more careful attention to the optical character of the amino acids found in hydrolysates. Whether traces of *d*-amino acids exist in native protein or arise by racemization during hydrolysis, some *d*-amino acids are usually present even in acid hydrolysates of proteins from normal tissues.

Chibnall and his associates (5) found appreciable concentrations of d(−)-glutamic acid in hydrolysates of edestin and wheat gliadin. Town (11) believes that the inactive glutamic acid which he isolated from the copper aspartate fraction of a gliadin hydrolysate is a racemic compound—as he puts it, a "definite hydrolysis product and not an artefact." If this is true, traces of *d*-amino acids in proteins must long have been overlooked.

In 1939 Dubos (12) isolated a soil bacillus which showed marked antagonistic activity toward every Gram positive microbial species tested. Upon autolysis, the bactericidal agent was liberated and could be precipitated from the autolysate by adding hydrochloric acid to a pH of 4.5. A single injection of 2 mg. of the precipitate intraabdominally into mice protected them against 10,000–100,000 fatal doses of several types of pneumococci and exerted a curative effect when

[1] Throughout this paper the prefix *d* or *l* refers to configuration, the (+) or (−) to direction of rotation (1). Since the commonly-occurring or "natural" amino acids all have the *l* configuration about the α carbon atom, they may be designated collectively as the *l*-amino acids, their optical "mirror images" as the "unnatural" or *d*-amino acids.

given after infection. Fractionation of the precipitate with various organic solvents led to the isolation of two crystalline polypeptides, one called Gramicidin because it acts selectively against Gram positive microorganisms, the other Tyrocidine to recall the fact that strains of the genus Tyrothrix (13) were among the first bacilli shown to possess bactericidal activity. The two products are of interest in this survey because they are said to contain *d*-amino acids. In hydrolysates of gramicidin the ammonia liberated by *d*-amino acid oxidase was equivalent to about 45 per cent of the α-amino-nitrogen; in hydrolysates of tyrocidine hydrochloride to about 20 per cent (14). Enough optically and analytically pure *d*-leucine was isolated to account for about half of the indicated *d*-amino acid content of gramicidin (15), but the nature of the other half was not determined. The configuration of the tryptophane isolated was of the *l*-type; alanine was estimated but not isolated in the free form. The optical properties of none of the amino acids in hydrolysates of tyrocidine have been determined. The spore-bearing bacilli which produce these polypeptides are so widely distributed in nature and so commonly found in cheese and other food products that, if the observations recorded are correct, traces of *d*-amino acids must certainly be encountered in the diet.

TABLE 1

Influence of configuration on taste

AMINO ACID	TASTE OF THE NATURAL ISOMER	TASTE OF THE UNNATURAL ISOMER	OBSERVER (16)
Asparagine	insipid	sweet	Piutti (1886)
Glutamic acid	unique	almost tasteless	Menozzi and Appiani (1894)
Leucine	flat, slightly bitter	strikingly sweet	Fischer and Warburg (1905)
Valine	slightly sweetish - bitter	decidedly sweet	Fischer (1906)
Serine	faintly sweet, stale aftertaste	strikingly sweet	Fischer and Jacobs (1906)
Histidine	bitter or tasteless	sweet (like cane sugar)	Abderhalden and Weil (1912)
Isoleucine	bitter	noticeably sweet	Ehrlich (1914)
Alanine	1 gm. as sweet as 0.73 gm. of cane sugar	1 gm. *dl* as sweet as 0.92 gm. of cane sugar	Heiduschka and Komm (1925)
Tryptophane	flat	very sweet	Author
Methionine	flat	sweet	Author
Phenylalanine	bitter	sweet, bitter after-taste	Author
Norleucine	slightly sweet	bitter	Abderhalden, Froehlich and Fuchs (1913)

The administration of mixtures of purified amino acids has now become feasible clinically and is often advantageous experimentally. Several amino acids can be prepared more readily and more cheaply by synthesis than by isolation. Separation of the natural isomer by resolution is usually both tedious and expensive. It may therefore be expedient to administer the racemic mixture, if the response of the subject to the *d* isomer is not objectionable.

A simple, yet convincing subjective proof that optical configuration affects physiological response may be had by noting the difference in taste of the antipodes of an amino acid. In table 1 are recorded descriptions of such tests taken from the literature or made by the author. The natural isomers usually taste flat, the "unnatural" *d*-amino acids usually sweet. Pasteur was interested enough in Piutti's report of the difference in taste of the asparagines to append a note commenting on the phenomenon as a response of the nerve endings to asymmetry in structure.

Demonstrations that resolution of racemic amino acids may occur in the animal were made in the rabbit by Wohlgemuth in 1905, since then with other animals and with other amino acids (17). As a general rule, the *d*-amino acid is somewhat more difficult to metabolize than the *l*. If the dosage of the racemic mixture is too small, both isomers may undergo oxidation; if too large, some of the *l* component will also be excreted.

Comparisons of rates of gastrointestinal absorption of the *l* and *dl* modifications of several amino acids determined in the rat by the Cori technique (18) seem to confirm the early observations of Dakin (19) that no selective absorption of the optical isomers obtains. However, rates of absorption for the free amino acid, the sodium salt, and the hydrochloride, may differ markedly. When the *dl* form is fed, each of the optical isomers is obviously supplied to the body only half as rapidly thereby as when either is fed alone.

The capacities of the *d* and *l* forms of the essential amino acids to support growth in the rat have recently been summarized by Rose (20). Both isomers of tryptophane, histidine, phenylalanine, and methionine are available for this purpose, but the "unnatural" forms of valine, leucine, isoleucine, lysine, and threonine are not. Similar tests on cystine show that only its natural form can be used for growth (21). The isomers of arginine have not been compared.

A few years ago Kotake, Ichihara and Nakata (22) reported that, unlike rats, mice could not grow on *d*-tryptophane. On the contrary, Totter and Berg (23) obtained growth, though at a some-

what slower rate than on the natural isomer; they found that *d*(−)-lysine could not be used and that the utilization of *d*-histidine was poor. Recently Bauer and Berg (24) have obtained slow growth in mice fed the 10 essential amino acids as the only source of protein nitrogen and have made comparisons, in diets containing 20 amino acids, between the isomers of essential amino acids (except arginine) not previously tested in that animal. In most instances the results were in harmony with those recorded for the rat. Comparisons of growth on the isomers of histidine fed in such diets showed interesting inconsistencies. When the vitamin B complex was supplied as a mixture of ryzamin B and crystalline thiamin, riboflavin, and nicotinic acid, good growth was obtained with *l*-histidine but little or none with the *d* form. When yeast Vitamin Harris concentrate was substituted, growth similar to that reported by Totter and Berg with that vitamin source occurred. In rats the purified amino acid diet promoted good growth, even with the more highly purified vitamin B complex. So far no satisfactory explanation has been found for these divergent results.

Promotion of growth by synthetic diets in which the unnatural isomer of an essential amino acid is fed can probably be regarded as presumptive evidence that conversion to the natural amino acid occurs. Direct proof of such conversion was first advanced by Conrad and Berg (25) who showed *a*, that when *d*-histidine was fed to rats in a diet deficient in *l*-histidine, more histidine was deposited in the tissues than could be accounted for by the histidine present at the outset of the study (as estimated from analyses of litter mates) and the traces of *l*-histidine in the food consumed during the growing period; *b*, that the histidine isolated as the methyl ester hydrochloride was essentially the optically pure *l*-isomer; and *c*, that since even traces of *d*-histidine added to the tissues before hydrolysis could be detected by the procedure adopted, *d*-histidine could not have been overlooked.

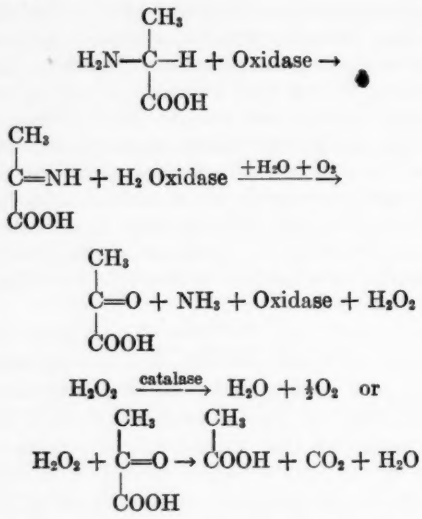
Using as a criterion the production of indole by a strain of B. coli which attacked *l*- but not *d*-tryptophane, Kotake and Goto (26) have shown that kidney and liver slices from the rat and the mouse convert *d*-tryptophane to *l*-tryptophane.

In the adult rat Ratner, Schoenheimer, and Rittenberg (27) have demonstrated the inversion of labeled *d*-leucine added to a complete stock diet. The carbon chain of the leucine was marked with deuterium, the amino group with heavy nitrogen. The leucine isolated from the tissues contained a considerable amount of the deuterium, but only a trace of the isotopic nitrogen. Apparently the latter had been lost by deamination and reamination. Failure of *d*-leucine to stimulate growth in young rats when it was fed in amino acid mixtures devoid of *l*-leucine (20) suggests that inversion fails to provide enough *l*-leucine to meet the greater demands of growth. It is interesting to note that *d*-leucine is rapidly oxidized by *d*-amino acid oxidase (28) and that the corresponding ketonic acid promotes growth when fed in diets deficient in leucine (20). Perhaps deamination is interrupted at some intermediate stage or the ketonic acid liberated in the process is more vulnerable to oxidation, demethylation, or conversion to acetone bodies than the ketonic acid fed preformed.

A few years ago duVigneaud and Irish (29) observed that the foreign *d*(−)-phenylaminobutyric acid was inverted in the dog and excreted as the acetylated *l*(+)-phenylaminobutyric acid. They suggested that oxidative deamination had produced the corresponding ketonic acid which had then condensed with ammonia and pyruvic acid to form an intermediate product readily convertible into the derivative excreted. Subsequent study of the process with *d*(−)- and *l*(+)-phenylaminobutyric acids containing N_{15} showed that in the inversion of the *d*(−) acid the amino nitrogen was probably removed completely, but that the *l*(+) acid could be acetylated without losing its original amino nitrogen.

Deaminating enzyme systems are found in the liver, the kidney, and the intestinal mucosa (30). Tissue slices and concentrated suspensions of ground tissue deaminize both *l*- and *d*-amino acids, the latter usually more readily than the former. Unlike the enzyme system which deaminizes the *l*-amino acids, the system affecting the *d*-amino acids can be extracted readily, is not destroyed by drying, or inhibited by octyl alcohol. The first system is known as *l*-amino acid deaminase; the second is now called *d*-amino acid oxidase because it not only deaminates the amino acids of the *d* series, but also demethylaminates their N-methyl derivatives and converts *d*-proline to α-keto-δ-amino-valeric acid without removing the amino group (31). With the free *d*-amino acids the reaction may be followed by measuring the oxygen absorbed, the ammonia produced, or even by isolating the ketonic acid formed. Production of hydrogen peroxide in the process (32) and reduction of methylene blue added to the system (33) support the thoery that the oxidation occurs as the result of dehydrogenation and hydrolysis. In the ordinary crude *d*-amino acid oxidase systems catalase decomposes the hydrogen peroxide formed; in purified systems which lack catalase, the hydrogen peroxide attacks the ke-

tonic acid (34). These reactions are represented below.



The *d*-amino acid oxidase system can be separated into two components—a specific protein and a prosthetic group. The protein component has been prepared in relatively pure form by denaturing the impurities and repeatedly precipitating the protein (34). The prosthetic group has been isolated and identified as flavin-adenine-dinucleotide (35). Mixture of the purified components produces a reconstituted enzyme system relatively free of impurities.

Riboflavin deficiency in the rat lowers the *d*-amino acid oxidase content of the liver and to a lesser extent that of the kidney; restoration may be effected by feeding riboflavin (36, 38). Feeding thyroid increases the protein, but not the flavin component (37, 38). Human blood cells can synthesize the latter from riboflavin *in vivo* and *in vitro* (39). Carnivorous animals have more *d*-amino acid oxidase in their liver and kidney tissues than omnivorous animals and omnivorous animals more than herbivorous (40).

The *d*-amino acids are not all oxidized at the same rate by *d*-amino acid oxidase. Using the reconstructed system, Klein and Handler (28) found that under similar conditions the *d* forms of alanine, leucine, isoleucine, norleucine, valine, methionine, proline, phenylalanine, and tyrosine were oxidized readily (94–60 per cent completely); those of tryptophane, serine, threonine, histidine, arginine, and aspartic acid were oxidized slowly (24–10 per cent); and those of lysine, cystine, and glutamic acid were not oxidized at all. With a similar system, Karrer and Frank (41) observed no oxidation of the *d* isomers of histidine, arginine, aspartic acid and serine. With less pure preparations, oxidation of *d*(−)-glutamic acid occurs.

Possibly other systems for deaminizing *d*-amino acids exist, but none have been demonstrated. Most of the evidence indicates that the *d*-amino acids cannot undergo appreciable transamination with α-ketoglutaric acid or α-ketosuccinic acid. Condensation with other ketonic acids as an intermediate step has not been excluded. Karrer and his associates (42) have recently shown that synthetic α, α′-imino-dipropionic acid (a type of compound which might result by conjugation of pyruvic acid with alanine, followed by reduction) can be oxidized by *d*-amino acid oxidase.

The influence of optical isomerism on the production of acetone bodies from the amino acids has been given only meagre attention. In liver perfusates Embden found a greater yield of acetone from *dl*- than from *l*-leucine, but suggested that the *l*-leucine may have been contaminated with norleucine (43). Studies of ketogenesis in liver slices from well-fed rats have shown no significant differences between the *l* and *d* forms of phenylalanine and the *l* and *dl* forms of tyrosine; *l*-leucine appeared less ketogenic than *dl*-leucine. In liver slices from starved rats only *dl*-leucine was appreciably ketogenic (44).

In the early demonstrations of glucose formation from amino acids in the phlorhizinized dog little distinction between optical modifications appears to have been made. Dakin reported that both *d*- and *l*-alanine were quantitatively transformed into glucose (45) and Greenwald that slightly less glucose was produced from *l*(+)- than from *d*(−)-norleucine (46). The few studies which present data for the storage of liver glycogen in rats or mice fed the natural and the racemic forms of the same amino acid indicate that the rate of deposition is usually faster on the natural isomer. Divergent results obtained in the earlier comparisons of *l*(+)- and *dl*-alanine (47, 48) can probably be traced to differences in the solutions fed and in the time of analysis after feeding. The only study on alanine affording direct comparison between the antipodes is a recent one on mice (49). Essentially the same amount of liver glycogen was ultimately formed from *dl*- as from *l* (+)-alanine, but much less was produced from *d*(−)-alanine. When the *d*(−)-alanine was fed alone, it was probably absorbed so rapidly as to exceed the capacity of the mouse to metabolize it; as a part of the *dl* mixture its rate of absorption was probably slow enough to allow its complete metabolism. *dl*-Alanine appears to be somewhat less effective than the *l*(+) isomer in reducing the urinary output of acetone bodies in fasted rats fed sodium acetoacetate (48); fasting ketosis seems to be lowered to approximately the same extent by either (50).

The natural isomers of aspartic and glutamic acid were somewhat superior to the *dl* mixtures,

both as glycogen formers and as ketolytic (or antiketogenic) agents (51); *l*-tyrosine caused appreciable storage of liver glycogen, *dl*-tyrosine much less (52). Both *dl*-threonine and *dl*-allothreonine increased the glycogen content of the liver and both decreased the ketonuria produced by feeding butyric acid (53).

l-Histidine produces considerable deposition of liver glycogen in 8 and 12 hours (54, 55). The few rats fed *d*-histidine seemed to show little storage in the same periods (54). If this difference in behavior can be substantiated in tests in progress the data will at least be consistent with the view that *l*(−)-histidine, but not its antipode, is converted catabolically by the liver enzyme, histidase, to *l*(+) glutamic acid (56).

The effect of configuration upon the response of the individual amino acids to numerous reactions of a more specific character can only be indicated in brief supplementary comments.

Histidine decarboxylase converts *l*-histidine, but probably not the *d* form, to histamine (57). In guinea pigs the injection of 0.1 gm. of *l*-histidine produces a significant increase of histamine in the lung, but repeated daily injections of 4 gm. of *d*-histidine are required to elicit a similar response (58). Growth can be obtained on histidine-deficient diets supplemented with imidazole-pyruvic (59) and imidazole lactic acids (60); growth on *d*-imidazole lactic acid is inferior to that on the *l* (61).

In the rabbit the pyrrole ring of tryptophane may be split prior to deamination to produce *l*-kynurenine from the *l*, and *d*-kynurenine from the *d* form (62); *l*-, but not *d*-, kynurenine can undergo deamination and ring closure to produce kynurenic acid (63, 64), but the process is rather inefficient. *l*-Kynurenine has been shown to function as a hormone in producing the brown pigment of the eye of Drosophila and certain other insects; *d*-kynurenine cannot be used similarly (65). The small amounts of kynurenic acid produced from *d*-tryptophane and from indolepyruvic acid in the rabbit, the dog, and the rat, are probably formed via *l*-tryptophane (66). Indole pyruvic acid (67, 68) and *l*(+)-, but not *d*(−)-, indolelactic acid can be utilized for growth (69). *d*-Tryptophane is said to yield indolepyruvic acid more readily than *l*-tryptophane (26).

The *d* form of phenylalanine produces the greater urinary output of phenylpyruvic acid in the rabbit (70, 71) and in mentally-defective humans manifesting the condition known as phenylpyruvic oligophrenia (72); administered phenylpyruvic acid is excreted readily (71, 72), but apparently also causes an increase of phenylalanine in the blood (73). In tissue slices both isomers of phenylalanine yield appreciable amounts of acetoacetic acid, but phenylpyruvic and phenyllactic acids do not (44); consequently, phenylalanine has been assumed to be converted to acetone bodies via tyrosine. The conversion of phenylalanine to tyrosine seems well established (74). Urinary excretion of ether-soluble phenols increases more after feeding *dl*- than after feeding *l*-tyrosine (75). Natural tyrosine appears to be oxidized by liver, but not by kidney suspensions (76), the *d* form by both. Surviving liver slices produce acetoacetic acid to approximately the same extent from *l*- and *dl*-tyrosine, *p*-hydroxy-phenyl-pyruvic acid, and homogentisic acid (44).

Comparisons of growth on suboptimum amounts of *l*- and *dl*-methionine in a diet containing 6 per cent of arachin suggest that natural methionine is somewhat more readily utilized (77). Both *d*- and *l*-homocystine promote growth (78) on cystine-deficient diets; if the diet is also deficient in methionine, homocystine is ineffective unless some transmethylating agent such as choline is also present (79). *d*-Methionine is reported to be as useful as *l*-methionine in preventing fatty infiltration in the liver of rats fed high fat-low protein diets (80). The stunting of growth produced by iodoacetic acid may be counteracted by *dl*-methionine, *dl*-homocystine, *l*-cystine, or *l*-cysteine, but not by *d*-cystine (81). Data on urinary sulfur distribution are similar for *l*- and *dl*-methionine and *l*-cystine (82), but suggest that *d*-cystine is oxidized with much greater difficulty (83). Extracts of the liver containing an enzyme known as desulfurase are said to liberate hydrogen sulfide much more readily from *l*- than from *d*-cysteine (84).

The influence of optical isomerism on the metabolism of arginine has had little study except with respect to arginase, which attacks both components of *dl*-arginine if enough time is allowed (85). Recently Hogan has shown that *d*(−)-lysine, in contrast to the *l*(+) form, has no capacity to cure the anemia produced by feeding deaminized casein (86).

In a brief survey references to many interesting papers and to certain aspects of metabolism must necessarily be omitted. Some pertinent data may have been overlooked because they were too well hidden in communications whose titles were not suggestive. Much recent foreign literature has, of course, been unavailable.

Still appropriate are the words which Dakin wrote twenty-nine years ago (87), "... it may be safely asserted that an accurate knowledge of what may happen to a food substance under any conditions, no matter how far removed from normal, is likely to be helpful in gradually filling in the intricate mosaic of metabolic reactions."

REFERENCES

(1) Fischer, E. and K. Raske. Ber. chem. Ges. **41:** 893, 1908. Wohl, A. and K. Freudenberg. Ber. chem. Ges. **56:** 309, 1923. (2) Pringsheim, H. Ztschr. physiol. Chem. **65:** 89, 1910. (3) Jacobs, W. and L. C. Craig. J. Biol. Chem. **110:** 521, 1935; S. Smith and G. M. Timmis. J. Chem. Soc. 396, 1937; G. Ivanovics and V. Bruckner. Ztschr. Immunitätsforschung **90:** 304, 1937; **91:** 175, 1937. (4) Kögl, F. Klin. Wchnschr. **18:** 801, 1939; Kögl and H. Erxleben. Ztschr. physiol. Chem. **258:** 57, 1939. (5) Chibnall, A. C., M. W. Rees, E. F. Williams and E. Boyland. Biochem. J. **34:** 285, 1940. (6) Kögl, F. and H. Erxleben. Ztschr. physiol. Chem. **264:** 198, 1940. (7) Graff, S., D. Rittenberg and G. L. Foster. J. Biol. Chem. **133:** 745, 1940. (8) Lipmann, F., O. K. Behrens, E. A. Kabit and D. Burk. Science **91:** 21, 1940. (9) Kögl, F., H Herken and H. Erxleben. Ztschr. physiol. Chem. **264:** 220, 1940. (10) Behrens, O. K., F. Lipmann, M. Cohn and D. Burk. Science **92:** 32, 1940. (11) Town, B. W. Biochem. J. **35:** 417, 1941. (12) Dubos, R. J. J. Exper. Med. **70:** 1, 1939; Proc. Soc. Exper. Biol. and Med. **40:** 311, 1939. (13) Dubos, R. J. and R. D. Hotchkiss, J. Exper. Med. **73:** 629, 1941. (14) Lipmann, F., R. D. Hotchkiss and R. J. Dubos. J. Biol. Chem. **141:** 163, 1941. (15) Hotchkiss, R. D. J. Biol. Chem. **141:** 171, 1941. (16) Piutti, A. Compt. Rend. Acad. Sc. **103:** 134, 1886; A. Menozzi and G. Appiani. Gazz. Chim. Ital. **24** (Part I): 370, 1894; E. Fischer and O. Warburg. Ber. Chem. Ges. **38:** 3997, 1905; E. Fischer. Ber. Chem. Ges. **39:** 2320, 1906; E. Fischer and W. A. Jacobs. Ber. Chem. Ges. **39:** 2942, 1906. E. Abderhalden and A. Weil. Ztschr. physiol. Chem. **77:** 435, 1912; F. Ehrlich. Biochem. Ztschr. **63:** 379, 1914; A. Heiduschka and E. Komm. Ztschr. angew. Chem. **38:** 291, 1925; E. Abderhalden, C. Froehlich and D. Fuchs. Ztschr. physiol. Chem. **86:** 454, 1913. (17) Wohlgemuth, J. Ber. Chem. Ges. **38:** 2064. 1905; see also references in C. P. Berg and M. Potgieter. J. Biol. Chem. **94:** 661, 1931–32. (18) Wilson, R. H. and H. B. Lewis. J. Biol. Chem. **84:** 511, 1929; C. P. Berg and L. C. Bauguess. J. Biol. Chem. **98:** 171, 1932; B. W. Chase and H. B. Lewis. J. Biol. Chem. **106:** 315, 1934. (19) Dakin, H. D. J. Biol. Chem. **4:** 437, 1908. (20) Rose, W. C. Physiol. Rev. **18:** 109, 1938. (21) du Vigneaud, V., R. Dorfmann and H. S. Loring. J. Biol. Chem. **98:** 577, 1932. (22) Kotake, Y., K. Ichihara and H. Nakata. Ztschr. physiol. Chem. **243:** 253, 1936. (23) Totter, J. R. and C. P. Berg. J. Biol. Chem. **127:** 375, 1939. (24) Bauer, C. D. and C. P. Berg. Unpublished data. (25) Conrad, R. M. and C. P. Berg. J. Biol. Chem. **117:** 351, 1937. (26) Kotake, Y. and S. Goto. Ztschr. physiol. Chem. **248:** 41, 1937. (27) Ratner, S., R. Schoenheimer and D. Rittenberg. J. Biol. Chem. **134:** 653, 1940. (28) Klein, J. R. and P. Handler. J. Biol. Chem. **139:** 103, 1941. (29) du Vigneaud, V. and O. J. Irish. J. Biol. Chem. **122:** 349, 1937–38. V. du Vigneaud, M. Cohn, G. B. Brown, O. J. Irish, R. Schoenheimer and D. Rittenberg. J. Biol. Chem. **131:** 273, 1939. (30) Neber, M. Ztschr. physiol. Chem. **240:** 59, 1936. (31) Krebs, H. A. Biochem. J. **29:** 1620, 1935; Enzymologia **7:** 53, 1939. (32) Keilin, D. and E. E. Hartree. Proc. Roy. Soc. (London) B. **119:** 114, 1936; F. Bernheim, M. L. C. Bernheim and A. G. Gillaspie. J. Biol. Chem. **114:** 657, 1936. (33) Bernheim, F. and M. L. C. Bernheim. J. Biol. Chem. **109:** 131, 1935. (34) Negelein, E. and H. Brömel. Biochem. Ztschr. **300:** 225, 1938–39. (35) Warburg, O. and W. Christian. Biochem. Ztschr. **298:** 150, 1938. (36) Axelrod, A. E., H. A. Sober and C. A. Elvehjem. J. Biol. Chem. **134:** 749, 1940. (37) Klein, J. R. J. Biol. Chem. **131:** 139, 1939. (38) Rossiter, R. J. J. Biol. Chem. **135:** 431, 1940. (39) Klein, J. R. and H. I. Kohn. J. Biol. Chem. **136:** 177, 1940. (40) Birkofer, L. and R. Wetzel. Ztschr. physiol. Chem. **264:** 31, 1940. (41) Karrer, P. and H. Frank. Helv. Chim. Acta. **23:** 948, 1940. (42) Karrer, P., H. Koenig and R. Legler. Helv. Chim. Acta. **24:** 861, 1941. (43) Embden, G. Beitr. chem. Physiol. u. Pathol. **11:** 348, 1908. (44) Edson, N. L. Biochem. J. **29:** 2498, 1935. (45) Dakin, H. D. Oxidations and reductions in the animal body. London, 2nd ed., 77, 1922. H. D. Dakin and H. W. Dudley. J. Biol. Chem. **17:** 451, 1914. (46) Greenwald, I. J. Biol. Chem. **25:** 81, 1916. (47) Wilson, R. H. and H. B. Lewis. J. Biol. Chem. **85:** 559, 1929–30. (48) Butts, J. S., M. S. Dunn and L. F. Hallman. J. Biol. Chem. **112:** 263, 1935–36. (49) MacKay, E. M., A. N. Wick and C. P. Barnum. J. Biol. Chem. **137:** 183, 1941. (50) Wick, A. N., E. M. MacKay, H. O. Carne and H. M. Mayfield. J. Biol. Chem. **136:** 237, 1940. (51) Butts, J. S., H. Blunden and M. S. Dunn. J. Biol. Chem. **119:** 247, 1937. (52) Butts, J. S., M. S. Dunn and L. F. Hallman. J. Biol. Chem. **123:** 711, 1938. Butts, J. S., R. O. Sinnhuber and M. S. Dunn. Proc. Soc. Exper. Biol. and Med. **46:** 671, 1941. (53) Hall, W. K., J. R. Doty and A. G. Eaton. Am. J. Physiol. **131:** 252, 1940–41. (54) Featherstone, R. M. and C. P. Berg. Unpublished data. (55) Butts, J. S. and L. F. Remmert. Fed. Proc. **1:** 103, 1942. (56) Edlbacher, S. and M. Neber. Ztschr. physiol. Chem. **224:** 261, 1934. E. Abderhalden and H. Hanson. Fermentforsch. **15:** 274, 1937. (57) Werle, E. and H. Herrmann. Biochem. Ztschr.

291: 105, 1937. (58) EDLBACHER, S., M. SIMON AND M. BECKER. Naturwiss. **28:** 411, 1940. (59) HARROW, B. AND C. P. SHERWIN. J. Biol. Chem. **70:** 683, 1926. (60) COX, G. J. AND W. C. ROSE, J. Biol. Chem. **68:** 781, 1926. (61) CROOKSHANK, H. R. AND C. P. BERG. Unpublished data. (62) KOTAKE, Y., JR. AND N. ITO. J. Biochem. (Japan) **25:** 71, 1937; C. P. BERG AND V. K. ROWE. Unpublished data. (63) KOTAKE, Y. AND J. IWAO. Ztschr. physiol. Chem. **195:** 139, 1931; R. BORCHERS AND C. P. BERG. Unpublished data. (64) KOTAKE, Y., JR. AND N. ITO. J. Biochem. (Japan) **26:** 161, 1937. (65) TATUM, E. L. AND A. J. HAAGEN-SMIT. J. Biol. Chem. **140:** 575, 1941. (66) BERG, C. P. J. Biol. Chem. **104:**373, 1934; L. C. BAUGUESS AND C. P. BERG. J. Biol. Chem. **104:** 691, 1934. (67) JACKSON, R. W. J. Biol. Chem. **84:** 1, 1929. (68) BERG, C. P., W. C. ROSE AND C. S. MARVEL. J. Biol. Chem. **85:** 219, 1929–30. (69) JACKSON, R. W. J. Biol. Chem. **73:** 523, 1927; K. ICHIHARA AND N. IWAKURA. Ztschr. physiol. Chem. **195:** 202, 1931; L. C. BAUGUESS AND C. P. BERG. J. Biol. Chem. **104:** 675, 1934. (70) KOTAKE, Y., Y. MASAI AND Y. MORI. Ztschr. physiol. Chem. **122:** 195, 1922. (71) CHANDLER, J. P. AND H. B. LEWIS. J. Biol. Chem. **96:** 619, 1932. (72) JERVIS, G. A. J, Biol. Chem. **126:** 305, 1938. (73) JERVIS, G. A.. R. J. BLOCK, D. BOLLING AND E. KANZE. J. Biol. Chem. **134:** 105, 1940. (74) MEDES, G. Biochem. J. **26:** 917, 1932; A. R. MOSS AND R. SCHOENHEIMER. J. Biol. Chem. **135:** 415, 1940. (75) FELIX, K. AND I. DIMARCO. Kolloid Ztschr. **89:** 293, 1939. (76) BERNHEIM, F. J. Biol. Chem. **111:** 217, 1935. (77) BENNET, M. A. Biochem. J. **33:** 885, 1939. (78) DYER, H. M. AND V. DUVIGNEAUD. J. Biol. Chem. **109:** 477, 1935. (79) DUVIGNEAUD, V., J. P. CHANDLER, A. W. MOYER AND D. M. KEPPEL. J. Biol. Chem. **131:** 57, 1939. (80) BEST, C. H. AND J. H. RIDOUT. J. Physiol. **97:** 489, 1940. (81) STEVENSON, E. S. AND A. WHITE. J. Biol. Chem. **134:** 709, 1940. (82) STEKOL, J. A. J. Biol. Chem. **109:** 147, 1935. (83) DUVIGNEAUD, V., H. A. CRAFT AND H. S. LORING. J. Biol. Chem. **104:** 81, 1934. (84) FROMAGEOT, C., E. WOOKEY AND P. CHAIX. Enzymologia **9:** 198, 1941. (85) FELIX, K. AND K. MORINAKA. Ztschr. physiol. Chem. **132:** 152, 1923; S. EDLBACHER AND A. ZELLER. Ztschr. physiol. Chem. **242:** 253, 1936. (86) HOGAN, A. G., E. L. POWELL AND R. E. GUERRANT. J. Biol. Chem. **137:** 41, 1941; A. G. HOGAN. Personal communication, 1941. (87) DAKIN, H. D. J. Biol. Chem. **14:** 321, 1913.

THE RELATION OF VITAMIN C TO THE METABOLISM OF THE AROMATIC AMINO ACIDS

ROBERT R. SEALOCK

Department of Vital Economics, University of Rochester, Rochester, N. Y.

The rôle of the water soluble vitamins in protein metabolism, although a comparatively new field of investigation, promises to be quite as interesting as the more clearly pictured rôle of these accessory factors in carbohydrate metabolism. Numerous brilliant researches of the past few years in the latter field demonstrate the relation of thiamine, riboflavin, nicotinic acid and possibly ascorbic acid, and thus furnish at least in part the chemical basis for the physiological functioning of these vitamins. Although amino acid catabolism is not as well defined chemically as is carbohydrate catabolism, even in our present state of knowledge there are adequate indications pointing to the same importance of the water soluble vitamins in the chemical reactions involved.

Evidence to support this viewpoint may be derived by turning to the experiments dealing with carbohydrate metabolism, for with the demonstration that pyruvic, oxaloacetic and ketoglutaric acids are component substances in the carbohydrate reactions (1) it is only necessary to recall that they are also products of protein metabolism, arising from deamination of the appropriate amino acid.

Indeed, it should be borne in mind that the pyruvic acid which appears in the urine during thiamine deficiency (2, 3) may be in part derived from the oxidative deamination of the amino acid, alanine. That thiamine as cocarboxylase may be essential to the decarboxylation of all keto acids arising from the amino acids is suggested by the demonstration of Closs and Fölling (4) that phenylpyruvic acid appears in the urine of thiamine deficient rats when extra phenylalanine is fed. The idea of the more general or inclusive functioning of thiamine is being regarded with more and more favor (5).

Additional evidence of the rôle of the water soluble vitamins in protein catabolsim is to be seen in the highly specific enzyme, *d*-amino acid oxidase (6), a riboflavin enzyme whose physiological function is not completely understood.

The anti-grey hair vitamin must also be of significance since melanin pigment which arises from amino acids is deposited under the partial control of this vitamin (7). This latter phase of protein metabolism is also associated in some way with the biochemistry of vitamin C since the latter will effectively remove the melanin pigmentation appearing in Addison's disease (8).

The additional importance of vitamin C is evident in recent developments pertaining to the aromatic amino acids phenylalanine and tyrosine. And it is this phase to which further discussion of the rôle of the water soluble vitamins in protein metabolism will be limited.

Phenylalanine and tyrosine, because of the presence of the benzene ring, possess special interest for the student of amino acid metabolism. That the para hydroxyl or phenolic group which represents the only difference between the two may be readily supplied by the animal body has been proved by Womack and Rose (9, 10) in experiments demonstrating the indispensable nature of the former and the dispensable nature of the latter. Their experiments likewise lead to the conclusion that the reverse reaction does not occur since tyrosine will not replace phenylalanine. Disruption of the aromatic ring may occur in the process of oxidation of the carbon structure as is apparent in the fact that both amino acids undergo conversion to acetone bodies (11) and thus are regarded as ketogenic amino acids. The mechanism for this conversion remains in considerable doubt although interesting hypotheses have been advanced (12). The importance of oxidative deamination with the subsequent production of the alpha keto and alpha hydroxy acids must not be overlooked as an important phase of their metabolism. The key position of the keto acid in the process of utilization is clearly evident from the researches of Knoop (13) and du Vigneaud and Irish (14) demonstrating the synthesis of acetyl phenylalanine. Of equal interest is the conversion of both compounds to homogentisic acid in the condition of abnormal metabolism known as alkaptonuria (15, 16), a condition which Papageorge and Lewis (17) have produced experimentally in the white rat by feeding extra phenylalanine. One other relation might be referred to, namely, conversion to melanin pigment by way of the intermediate, 3,4-dihydroxyphenylalanine and the successive 5,6-dihydroxyindoles as described by Raper and his associates (18).

With this brief survey, we turn to the immediate subject of this review, a subject which has developed simultaneously in two different laboratories and from two different approaches. In our own laboratory the relation of vitamin C to melanin pigmentation was under investigation. In these experiments the feeding of extra tyrosine to vitamin C deficient guinea pigs resulted not only in an increased vitamin C requirement but also in the excretion of a urine which turned black upon exposure to air. By means of the usual qualitative and quantitative tests the presence of homogentisic acid was demonstrated and confirmation was obtained by isolation of a derivative, the dibenzoylhomogentisamide. Of even greater interest was the finding that the compound was present only in the urine of the vitamin deficient guinea pig and not at all when an adequate amount of ascorbic acid was administered (19, 20).

Although homogentisic acid was the first compound identified, the fractionation procedure for its isolation clearly indicated the occurrence of other compounds not usually found in the urine. The principal one of these proved to be para-hydroxyphenyl-pyruvic acid which arises by oxidative deamination of tyrosine. Later relatively small amounts of the corresponding alpha hydroxy acid and free tyrosine were also obtained.

The daily urinary excretion of these compounds was followed by means of the Briggs reaction for homogentisic acid, the 2,4-dinitrophenylhydrazine method for the keto acid and the Millon's reaction for the total tyrosyl values, the latter method including the alpha keto and hydroxy acids. While the methods leave much to be desired in regard to specificity, the proper use of calibration factors makes them fairly suitable for these experiments. This is particularly true since the differences between control and experimental periods are comparatively large. With their use it was easily demonstrated that none of the above metabolites are present when adequate vitamin C is given (21). Or in other words, the complete metabolism of phenylalanine and tyrosine by the guinea pig is dependent upon a certain minimal supply of the antiscorbutic substance.

The newly found role of ascorbic acid might have remained of purely academic interest had not Levine, Marples and Gordon simultaneously observed essentially the same phenomenon in the premature infant (22, 23, 24). These investigators at the Cornell University Medical College in the course of studies of the urinary excretion of creatinine observed false positive Jaffe reactions in the urines of the infants fed cow's milk. Suitable analytical procedures proved the presence of the alpha hydroxy and alpha keto acids derived from tyrosine. Administration of vitamin C resulted in the removal of the compounds from the urine. Furthermore, the vitamin deficiency during the period of metabolite excretion was confirmed by blood analyses showing negative or extremely low vitamin C values. The same diet fed to full term infants caused the excretion of

these compounds only when extra tyrosine or phenylalanine was added.

Two points of difference exist in the results from the two laboratories and with the two species. In the first place, the premature infants do not excrete appreciable amounts of homogentisic acid and in the second place they excrete relatively greater amounts of hydroxy acid than of keto acid as compared with the guinea pig. Such differences, however, do not detract from the essentially similar relationship disclosed, but represent minor phases which will no doubt be clarified in future experiments.

Before proceeding to other aspects of the question, we might examine in more detail the picture obtained with the vitamin C deficient guinea pig. When 0.5 to 1.0 gram of extra l-tyrosine is fed there occurs a marked excretion in 48 hours, and by three or four days 50 to 100 per cent of the supplemental amino acid appears in the urine in the form of abnormal metabolites. After establishment of an average excretion level, vitamin C may be administered and the urines analysed as in the previous period. With a daily dose of 10 mgm. of l-ascorbic acid, the urines show within 24 to 72 hours only the usual trace of compounds. Doses of 5 mgm. prove almost as effective. In fact, it appears that 5 mgm. of the vitamin is entirely adequate to counteract the ingestion of 0.5 gram of phenylalanine or tyrosine and also to supply the other vitamin C requirements of 300 to 400 gram guinea pigs. This ratio of 5 mgm. to 500 mgm. emphasizes the catalytic role of vitamin C in this relation.

The feeding of comparable amounts of phenylalanine does not give rise in the vitamin C deficient animal to phenylpyruvic acid but rather to the keto acid corresponding to tyrosine. This was demonstrated in our own experiments by the fact that the 2,4-dinitrophenylhydrazone isolated during the vitamin C free period proved to be that of the phenolic keto acid rather than of phenylpyruvic acid. This is shown in the accompanying figure 1 where the tyrosine value (Millon's) consists almost entirely of the keto acid. The remainder represents a small amount of the hydroxy acid and still less of free tyrosine. The figure also shows the homogentisic acid which arises from phenylalanine as well as from tyrosine feeding.

With the excretion of phenylalanine as a tyrosine derivative by the vitamin C deficient animal, it was of particular interest to determine the fate of phenylpyruvic acid, the primary oxidation product of phenylalanine. Subsequent feeding of phenylpyruvic acid resulted in its ready conversion and excretion in the urine as homogentisic acid and the tyrosine keto acid. Or in other words, this compound likewise was oxidized to a para hydroxy derivative, although the actual amount of metabolites in the urine was less than when phenylalanine was fed. If one may conjecture on the basis of comparative yields, it seems possible that the amino compound is more important in the pathway of conversion to tyrosine than is the keto acid as Embden and Baldes (25) originally suggested. When *p*-hydroxyphenylpyruvic acid is fed, contrary to what one might have predicted, numerous experiments demonstrated that it is metabolised fairly completely by the vitamin C deficient guinea pigs. Almost insignificant amounts of homogentisic acid and the fed compound were excreted in the urine even though the animal was continued on the deficient diet until gross symptoms of scurvy appeared. These results may prove of consider-

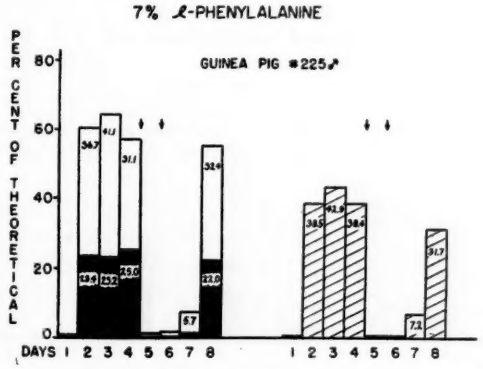


Fig. 1. Excretion of homogentisic acid (solid columns) α-keto acid (clear columns), and tyrosyl metabolites (cross-hatched columns) by vitamin C-deficient guinea pigs. The latter values are calculated as tyrosine. The arrows indicate the administration of 10 mgm. of *l*-ascorbic acid. The per cent of theoretical is calculated from the amount of supplement eaten.

able value in further study of the mechanism of vitamin C action and at least they emphasize the importance of the alpha amino group of tyrosine. As a working hypothesis we suggest that the point of action of the vitamin lies between the phenylalanine molecule and the production of the keto acid of tyrosine.

Chandler and Lewis (26) have placed similar emphasis upon the intact side chain in connection with complete oxidation of the benzene ring of phenylalanine. However, the experiments of Lewis and coworkers (26, 27) as well as those of Dakin (12) differ from the vitamin C studies in that no evidence for conversion of phenyl to hydroxyphenyl compound was obtained with rabbits (three kilos) whereas Kotake (28) observed conversion with larger amounts of amino acid and slightly smaller members of the same

species. Since the very young rabbit is somewhat susceptible to vitamin C deficiency, might not repetition of the phenylalanine experiments with weanling animals lead to results more like those obtained with the guinea pig and premature infant?

Very early in the investigations involving this new relationship of ascorbic acid, it became necessary to prove that the phenomenon observed was truly a function of the antiscorbutic compound. For example, ascorbic acid is a strongly reducing substance, which suggested that other reducing substances might also cause the disappearance of the urinary metabolites. Rather than test a large number of other compounds such as cysteine or glutathione, we have depended upon the asymmetric properties of the vitamin molecule and the relation of these to vitamin potency. For

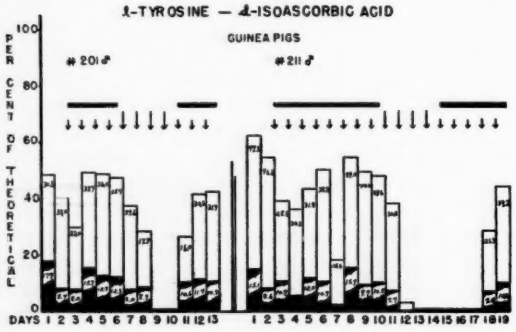

l-TYROSINE — d-ISOASCORBIC ACID
GUINEA PIGS
#201 ♂
#211 ♂
PER CENT OF THEORETICAL
DAYS 1 2 3 4 5 6 7 8 9 10 11 12 13
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19

Fig. 2. Metabolite excretion, and *d*-isoascorbic acid with 5 per cent tyrosine diet. Homogentisic acid, solid columns; *p*-hydroxyphenylpyruvic acid, clear columns; 10 mgm. of *l*-ascorbic acid, arrows; 10 mgm. *d*-isoascorbic acid, arrows with a bar above.

example, the diastereoisomer, d-isoascorbic acid, possesses identical reducing properties but only one twentieth as much antiscorbutic activity as the natural l-ascorbic acid. In a series of experiments, the effect of giving 10 mgm. of the unnatural compound instead of the 10 mgm. of the natural vitamin was tested. While this dose of the latter material had always proved effective, the figure shows the ineffectiveness of the isomer. It is also evident that replacement with the natural vitamin produces the usual effect and that the ineffectiveness of the d-isoascorbic acid is confirmed in the third phase of the experiment. But since this isomer is one twentieth as active as the vitamin C in preventing scurvy the dose was increased 20 times, 200 mgm. being given each day. Within the usual time the metabolite excretion was completely eliminated. In the face of this evidence it seems entirely justified to conclude that the action of vitamin C is truly inherent in the antiscorbutic activity of the molecule rather than in some non-specific reduction property.

Levine, Gordon and Marples have also presented confirmatory evidence in this direction and have shown the ineffectiveness of the B vitamins, choline, rice polishings, yeast and liver extract.

Equally as important as vitamin specificity is the question: Does incomplete metabolism of other amino acids occur in vitamin C deficiency? Of several tested, none have yielded keto acids or similar products in the urine under the conditions of the tyrosine experiments. However, in the process of testing glutamic acid, an interesting relationship was observed. When extra glutamic acid failed to yield urinary metabolites it was replaced with extra tyrosine for control purposes. Instead of the customary prompt appearance of aromatic compounds in the urine an unusual delay occurred.

The suspicion that the previous feeding of glutamic acid was in some way responsible was tested by administering single doses of glutamic acid to tyrosine fed vitamin C deficient guinea pigs. Such a procedure resulted in a marked decrease in the tyrosine metabolites in the succeeding 24 hour urine sample and a lesser effect if repeated after several days had intervened. Since glutamic acid may be converted to ketoglutaric acid, it seemed possible that the Szent-Györgyi-Krebs dicarboxyllic acid cycle might be operating in conjunction with vitamin C present in the tissues. The testing of this hypothesis was accomplished by using instead of glutamic acid other constituents of the catalytic cycle. For control purposes other compounds similar in structure but not identified with the first group in tissue oxidation were used. For example, the experiments with fumaric acid were compared with experiments employing the geometric isomer, maleic acid. In general, the compounds of either group were quite as effective as glutamic acid. Since their one property in common is that of acidity, further controls were instituted by feeding these acids with sodium bicarbonate and as the mono and disodium salts. In the order named this represents the administration of an increasing ratio of base to acid, and in this order less and less effect upon tyrosine metabolites was obtained. The accompanying figure 3 summarizes these results with all acidic compounds used, showing the change in per cent of theoretical yield of keto acid in the 24 hour period following administration of the test substance. This conclusion that the effect produced is due primarily to the acidity of the compounds used is supported by the results obtained with the common acidifying agent, ammonium chloride and by the finding

that the inclusion of extra sodium bicarbonate in the diet mixture tends to decrease the effectiveness of single doses of ascorbic acid (fig. 4).

The possibility of the Szent-Györgyi-Krebs system also operating is not entirely eliminated The prominence of the acid action requires a large number of carefully balanced experiments in the future before a decision may be made with certainty.

Fortunately, an explanation for the remarkable effect of acidification in vivo is available in the work of Hawley, Stephens and Daggs (29, 30) demonstrating mobilization of tissue vitamin C by administration of ammonium chloride to man or guinea pigs. The reverse situation was obtained by these authors with sodium bicarbonate. It is thus conceivable that in our experiments the acid mobilization moves to the liver (or kidneys) just enough reserve ascorbic acid to cause more complete metabolism of that day's tyrosine. From the standpoint of practical nutrition, the more recent results confirm entirely the statement of Hawley and coworkers concerning the importance of the acid-base balance to vitamin C saturation.

Homogentisic acid deserves some special consideration for as far as vitamin C is concerned there are apparent anomalies. The discrepancy between the experiments with the guinea pigs and the premature infants has been mentioned. In this connection, the paper of Papageorge and Lewis (17) indicating the necessity of a minimal intake of 300 mgm. of phenyalanine per 100 gm. of rat per day should be cited. In earlier experiments we observed homogentisic acid excretion in every instance when 150 to 330 mgm. of extra tyrosine per 100 grams of animal was fed. However, this did not rule out the possibility of a minimal intake being necessary even in this species which is unable to elaborate ascorbic acid as does the rat. More recently this point has been tested. With daily intakes of tyrosine of approximately 100 mgm. per 100 gram of body weight only small amounts or traces of homogentisic acid were observed, although the excretion of the keto acid was maintained at the usual level. Continuation of these experiments will no doubt establish a necessary minimal intake more definitely. If these findings may be extended to another species susceptible to vitamin C deficiency the failure of the premature infant to exhibit alkaptonuria is entirely understandable. In fact, Levine, Marples and Gordon (23) report continued total intakes below 150 mgm. per 100 gram in their discussion of this very point. Their administration of larger amounts (200 mgm. per 100 grams) was in single doses only and, of course, did not fulfill, as they indicate, the second requirement of prolonged administration stipulated by Papageorge and Lewis.

There are other complications to the problem, for Cook (private communication) of the University of St. Andrews has confirmed the homogentisic acid excretion of scorbutic guinea pigs but found no appreciable amounts of ketonic acids. In his experiments dl-phenylalanine was fed with a different basal diet than ours.

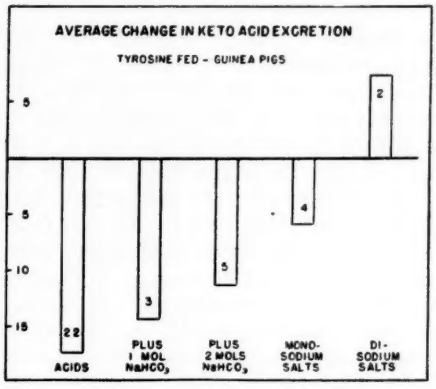


Fig. 3. Summary of effect of dicarboxyllic acids. Change in per cent yield of *p*-hydroxyphenylpyruvic acid in 24 hours after 3.3 millimols of test substance. Basal diet—vitamin C-free + 0.5 gram *l*-tyrosine daily. Number of experiments ndicated in each column.

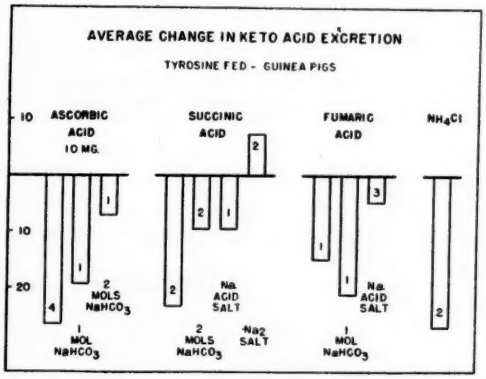


Fig. 4. Effect of acid and alkali. Change in per cent yield of *p*-hydroxyphenylpyruvic acid in 24 hours after 3.3 millimols of test substance. Basal diet—vitamin C-free + 0.5 gram *l*-tyrosine daily. Number of experiments indicated in each column.

The question of homogentisic acid cannot be dropped without reference to the ineffectiveness of ascorbic acid in checking alkaptonuria in man (31, 32, 33). Since, in our experiments with Galdston and Steele, even 4.0 grams of the vitamin per day caused no change in the level of excretion, the hereditary condition perhaps should be re-

garded as a result of the absence of some factor other than ascorbic acid. This factor may even be the protein part of a specific enzyme rather than a relatively simple prosthetic group.

In the foregoing discussion a specific property of the antiscorbutic vitamin, that is, its importance to the metabolism of the aromatic amino acids, phenylalanine and tyrosine, has been reviewed. The relations disclosed may or may not aid in clarifying the fundamental biochemistry of scurvy, but they do support the original premise that the water soluble vitamins are important factors in the chemical reactions of protein metabolism.

REFERENCES

(1) Elliot, K. A. C. Physiol. Rev. **21:** 267, 1941. (2) Harper, H. A. and H. J. Deuel, Jr. J. Biol. Chem. **137:** 233, 1941. (3) Shils, M. E., H. G. Day and E. V. McCollum. J. Biol. Chem. **139:** 145, 1941. (4) Closs, K. and A. Fölling. Ztschr. physiol. Chem. **254:** 258, 1938. (5) Green, D. E. Mechanisms of biological oxidation. London, Cambridge Univ. Press, 1940. (6) Krebs, H. A. Biochem. J. **29:** 1620, 1935. (7) Morgan, A. F. and H. D. Simms. J. Nutrition **19:** 233, 1940. (8) Szent-Györgyi, A. Science **72:** 125, 1930. (9) Womack, M. and W. C. Rose. J. Biol. Chem. **107:** 449, 1934. (10) Rose, W. C. Physiol. Rev. **18:** 109, 1938. (11) Baer, J. and L. Blum. Arch. f. exper. Path. u. Pharmakol. **56:** 92, 1907. (12) Dakin, H. D. Oxidations and reductions in the animal body. London, Longmans, Green and Co., 1922. (13) Knoop, F. and J. G. Blanco. Ztschr. physiol. Chem. **146:** 267, 1925. (14) du Vigneaud, V. and O. J. Irish. J. Biol. Chem. **122:** 349, 1938. (15) Wolkow, M. and E. Baumann. Ztschr. physiol. Chem. **15:** 228, 1891. (16) Falta, W. and L. Langstein. Ztschr. physiol. Chem. **37:** 513, 1903. (17) Papageorge, E. and H. B. Lewis. J. Biol. Chem. **123:** 211, 1938. (18) Raper, H. S. Biochem. J. **21:** 89, 1927. (19) Sealock, R. R. and H. E. Silberstein. Science **90:** 517, 1939. (20) Sealock, R. R. and H. E. Silberstein. J. Biol. Chem. **135:** 251, 1940. (21) Sealock, R. R., J. D. Perkinson, Jr. and D. H. Basinski. J. Biol. Chem. **140:** 153, 1941. (22) Levine, S. Z., E. Marples and H. H. Gordon. Science **90:** 620, 1939. (23) Levine, S. Z., E. Marples and H. H. Gordon. J. Clin. Investigation **20:** 199, 1941. (24) Levine, S. Z., E. Marples and H. H. Gordon. J. Clin. Investigation **20:** 209, 1941. (25) Embden, G. and K. Baldes. Biochem. Ztschr. **55:** 301, 1913. (26) Chandler, J. P. and H. B. Lewis. J. Biol. Chem. **96:** 619, 1932. (27) Shambaugh, N. F., H. B. Lewis and D. Tourtellotte. J. Biol. Chem. **92:** 499, 1931. (28) Kotake, Y., Y. Masai and Y. Mori. Ztschr. physiol. Chem. **122:** 195, 1922. (29) Hawley, E. E., J. P. Frazier, L. L. Button and D. J. Stephens. J. Nutrition **12:** 215, 1936. (30) Hawley, E. E., R. G. Daggs and D. J. Stephens. J. Nutrition **14:** 1, 1937. (31) Monsonyi, L. Presse med. **47:** 708, 1939. (32) Diaz, C. J., H. C. Mendoza and J. S. Rodriquez. Klin. Wchnschr. **18:** 965, 1939. (33) Sealock, R. R., M. Galdston and J. M. Steele. Proc. Soc. Exper. Biol. and Med. **44:** 580, 1940.

The American Society for Pharmacology and Experimental Therapeutics

Symposium on Deficiency Diseases

April 3, 1942

TOM D. SPIES, Chairman

A true concept of the value of food as a therapeutic agent has been gained only in recent years. Certain we are that every living cell in the human body requires specific nutrients in order to function normally. Respiration and growth of these cells require the synthesis of complex substances from simple ones. After many months or years in which the level of these essential substances is decreased in the body, symptoms may develop, and eventually structural changes appear and finally a clinical diagnosis of a deficiency disease can be made by the trained observer.

The essence of treatment is early and accurate diagnosis. The physician is badly in need of help from pharmacologists and other scientists so that his medical history and physical examination may be interpreted properly in the light of physiology, chemistry and pharmacology. It is fallacious to regard deficiency diseases as rare, and I feel that the pharmacologists and others can make a real contribution to national welfare in the time of this crisis by putting their shoulders to the wheel.

SOME SPECIFIC PHYSIOLOGICAL DISTURBANCES INDUCED BY MARGINAL VITAMIN DEFICIENCIES (C AND B_1)[1]

C. G. KING

Professor of Chemistry, University of Pittsburgh

There is good agreement among those who are actively working in the related fields of study in America that moderate degrees of malnutrition are fairly common in nearly all age groups, economic levels and geographical areas of the United States and Canada. The strange feature of the present picture is that so many professional and well educated people have not been convinced that malnutrition is a significant factor in keeping our national health and general welfare below their best levels.

Purely from a public health point of view, I think it is clear that there is need for increased support of basic research programs that are designed solely to discover and establish general principles in the science of nutrition. But there is also a clear need for two additional types of research:

First, to discover in experimental animals the kinds of disturbances that arise from moderate degrees of malnutrition; and

Second, careful clinical experimentation, with rigid controls, far better than those generally provided for, to discover the contribution that *good nutrition at all stages of development* can make to human health (8). The present paper deals primarily with the first of these two types of special study. The major research interest of our group in Pittsburgh has been in the more theoretical types of work, however, as chemists.

About two years ago, Mr. Robert Patton, a graduate student in the Department of Psychology, and his major advisor, Dr. Harry W. Karn, asked me to collaborate with them in preliminary studies of convulsive seizures in albino rats. One group of animals was very sensitive to convulsive (epileptoid) seizures when exposed to the noise of an electric buzzer. A second group was not so sensitive. The only known difference in the background of the two groups was that the less sensitive group had received nominal amounts of supplementary foods in addition to their regular dog chow (Purina). Preliminary tests indicated that both vitamin B_1 deficiency and inanition were causative agents in establishing the sensitivity. Later tests, carried out repeatedly and with fairly large groups of animals, confirmed the first findings and showed that other vitamins in the B-complex were also important factors in protecting against the seizures (16, 17, 9 and 2).

The data given in table 1, and figures 1 and 2 serve to illustrate the nature of the findings. Chick and her associates (2) have described similar epileptoid seizures that resulted from prolonged vitamin B_6 (pyridoxine) deficiency. More recently, Lepkovsky, Krause and Dimick (11) reported comparable findings, observed only in older animals that were 4.5 to 9 months of age. Maier has ascribed comparable seizures to mental conflict situations (14), without regard to noise or nutritional factors, but neither our laboratory nor some others (15) could confirm the "mental conflict" relationship.

Spies (23), Wilder (22), Jolliffe (7) and others have observed poor nerve function and excessive irritation by noise in clinical vitamin B_1 deficiency, but apparently a thorough study of the

TABLE 1

Effect of thiamin on convulsive seizures in rats subjected to auditory stimulation

GROUP	DAILY SUPPLEMENT	NUMBER OF DAYS TESTED	TOTAL NUMBER OF SEIZURES PER WEEK FOR 8 ANIMALS		TOTAL NUMBER OF SEIZURES (TOTAL NUMBER POSSIBLE FOR 8 RATS, 112)
			1st week	2nd week	
Experimental....	20γ of thiamin*	14	44	38	82
Control.....	None	14	56	55	111

* 20γ per milliliter, fed by pipette. (1γ = 0.001 mg.)

Effect of vitamins B_1, B_2, and B_6 on convulsive seizures in rats subjected to auditory stimulation

GROUP	DAILY SUPPLEMENT	NUMBER OF DAYS TESTED	NUMBER OF ANIMALS	TOTAL NUMBER OF SEIZURES (TOTAL NUMBER POSSIBLE FOR 8 RATS, 80)
Experimental.....	20γ of vitamins B_1, B_2, B_6*	10	8	48
Control.....	None	10	8	80

* 20γ each per milliliter, fed by pipette.

(From reference 17, reproduced by permission of the Editor.)

[1] The studies are a part of a research program supported by grants from the Buhl Foundation of Pittsburgh and the Williams-Waterman Fund of the Research Corporation of New York. Contribution no. 466 from the Department of Chemistry, University of Pittsburgh.

problem on a quantitative basis has not been possible up to the present time. The work with rats has at least suggested, however, that studies of this nature would be of interest to those in industry and in military organizations where the performance of men under strain and during exposure to excessive noise is of critical importance.

In our studies of the rôle of vitamin C (ascorbic acid) in animal tissues, one of the most interesting types of observation deals with the regulatory or balancing rôle that the vitamin plays in a great variety of chemical reaction systems in which enzymes are involved. When there is no regular intake of the vitamin, the rate of depletion from specific tissues varies greatly, and it appears reasonably certain that body functions fluctuate with the changes in vitamin intake. The glandular tissues tend to conserve their reserves in varying degrees, but there is no capacity for prolonged storage in any part of the body. In the growing teeth and bones, structural changes become evident very quickly, but in most other tissues the observable changes are more gradual. By chemical analysis or measure of tissue enzymes, it is possible to demonstrate a gradual transition in the activity of a great variety of enzymes. There are parallel, but slower, changes in general tissue composition (table 2).

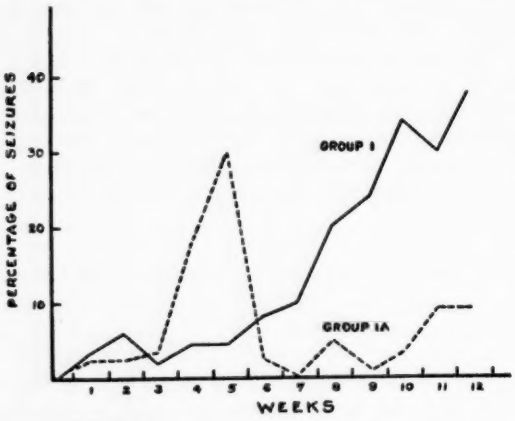


Fig. 1. Average percentage of seizures in vitamin B_1 deficient animals (group 1) and in their paired feeding controls (group 1A). See * reference 17 (reproduced by permission of the Editor).

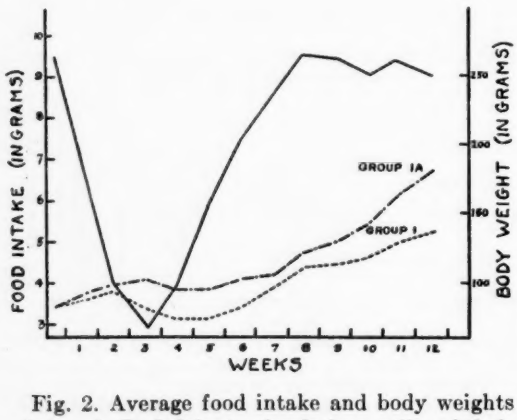


Fig. 2. Average food intake and body weights of vitamin B_1 deficient animals (group 1) and their controls (group 1A).

Lund and Cranden (13) and others have shown that obvious disturbances in human health follow only slowly when the intake of vitamin C is extremely low, but the more extensive chemical and histological studies with guinea pigs point toward significant changes within a short period (1, 6). Shwachman (19) and Gould (4) have proposed the decrease in plasma phosphatase as a basis for clinical diagnosis of moderate vitamin C deficiency. The method offers at least a good supplementary test, even though a number of metabolic disturbances also affect the plasma phosphatase activity to a marked degree. It is evident that marginal vitamin C deficiency should be considered, in turn, when using the blood phosphatase test to diagnose other conditions such as rickets.

TABLE 2

Effect of vitamin C depletion and recovery on glucose tolerance of guinea pigs

DEPLETION PERIOD	MGM. GLUCOSE PER 100 CC. BLOOD AFTER				NO. OF ANIMALS	AVERAGE WEIGHT	MINIMUM AND MAXIMUM WEIGHTS
	Fasting	40 min.	80 min.	120 min.			
days						*gm.*	*gm.*
Controls (2 mg. per day)	101±0.9*	149±3.9	141±3.7	95±1.4	15	583	480–718
10	104±1.4	169±3.3	161±4.7	108±3.0	14	615	470–740
15	109±1.6	176±4.3	177±4.7	131±2.9	15	593	437–747
20	112±1.3	185±4.2	191±5.1	150±5.4	15	507	337–615
RECOVERY PERIOD							
10	101±1.5	163±4.4	144±6.0	109±3.4	13	499	327–707
15	99±0.9	156±2.1	139±1.6	100±1.4	13	528	347–720

(From reference 20, reproduced by permission of the Editor.)

In parallel with the onset of a deficiency, there are gradual changes in the general physiology. Respiration is increased (3), sugar tolerance is lowered (20), nitrogen clearance is altered, as shown by Sealock for amino acids (18), and there is a general lowered resistance to tissue injury by chemicals and biological toxins. It is also clear from the work in a number of laboratories in addition to our own, that metabolic disturbances can

lead to a greatly increased demand for ascorbic acid in the tissues. In other words there is a two-way relationship: (a) a lowered intake of the vitamin robs the tissues of their normal resistance, and (b) injuries such as poisoning or infection cause excessive loss of the limited stores of the vitamin that are available. This type of dual relationship or vicious circle can be demonstrated especially well by the use of diphtheria toxin, and by chemical titration to measure the vitamin in parallel with histological studies of the teeth (10) (tables 3 and 4).

The paper by Urban and Peugnet (21) and a recent note by Linstead and Kraye (12) point most interestingly toward an important rôle played by ascorbic acid in regulating heart action. Of special interest to pharmacologists too, perhaps, is the relationship shown by Green and Musulin (5) and others, that decreased ascorbic acid renders guinea pigs more susceptible to the effect of barbiturates. Clinically, however, we did not find that the barbiturates affected urinary excretion of the vitamin.

I am inclined to think that vitamin C deficiencies are most common and perhaps most injurious at the two extremes of life. Many professional people and laymen still do not fully appreciate the fact that even raw cow's milk contains only about one-fourth as much vitamin C as is normally contained in mother's milk. Hence the risk of moderate deficiencies among infants and small children is fairly great before they have reached the stage of eating generous amounts of good antiscorbutic foods. In a comparable fashion, many old people become finicky in their eating habits and do not consume liberal quantities of antiscorbutic foods.

SUMMARY. The author suggests that the more recent quantitative studies of vitamin deficiencies point with reasonable certainty toward the conclusion that moderate and transient deficiencies are probably significant factors in relation to health and behavior. The levels of intake that are high enough to ward off external signs of malnutrition should not be accepted as satisfactory standards. Even though much more research is needed to provide a good basis for evaluating marginal types of deficiency, the trend of the evidence seems unmistakable. The table of "Recommended Allowances" prepared by the National Research Council as a guide for "good nutrition" represents a great advance in viewpoint as compared to an earlier tendency of scientific groups to publish only tables of "protective" levels of intake.

TABLE 3

Tissue changes coincident with scurvy

MEASURED	CHANGE WITH SEVERE VITAMIN C DEFICIENCY
Liver esterase	−65%
Liver phosphatase	−12
Brain phosphatase	−16
Muscle succinic dehydrogenase	−65
Heart succinic dehydrogenase	−20
Heart cytochrome oxidase	−44
Oxygen consumption of liver	+40
Oxygen consumption of guinea pig (ref. 3)	+24

(From reference 6, reproduced by permission of the Editor.)

TABLE 4

Effect of diphtheria toxin on the vitamin C content of guinea pig tissues

Feeding level of vitamin 0.5 mgm. per day, total toxin 1.1 M.L.D (2 injections)

TISSUE	NUMBER OF ANIMALS	GROUP	WEIGHT OF TISSUE	VITAMIN CONTENT	TOTAL VITAMIN	LOSS OF VITAMIN
			grams	*mgm. per gram*	*mgm.*	*per cent*
Adrenals	16	Controls	0.29	0.076	0.022	
	16	Toxin	0.46	0.031	0.014	36
Pancreas	16	Controls	1.07	0.022	0.024	
	16	Toxin	0.77	0.019	0.015	38
Kidneys	16	Controls	3.44	0.021	0.072	
	16	Toxin	3.50	0.016	0.056	22
Liver	16	Controls		0.034		
	16	Toxin		0.038		12 (gain)

(From reference 20, reproduced by permission of the Editor.)

REFERENCES

(1) BESSEY, O. A. AND C. G. KING. J. Biol. Chem. **103:** 687, 1933; C. G. KING. Physiol. Rev. **16:** 238, 1936. (2) CHICK, H., M. M. EL SADR AND A. V. WORDEN. Biochem. J. **34:** 594, 1940. (3) FIDLAR, E., M. SHEPPARD AND E. W. MCHENRY. Biochem. J. **33:** 344, 1939. (4) GOULD, B. S. AND H. SHWACHMAN. Am. J. Physiol. **135:** 485, 1942. (5) GREEN, M. W. AND R. R. MUSULIN. J. Am. Pharm. Assoc. **30:** 613, 1941. (6) HARRER, C. J. AND C. G. KING. J. Biol. Chem. **138:** 111, 1941. (7) JOLLIFFE, N., R. GOODHART, J. GENNIS AND J. K. CLINE. Am. J. Med. Sci. **198:** 198, 1939. (8) JOLLIFFE, N., J. S. MCLESTER AND H. C. SHERMAN. J. A. M. A., **118:** 944, 1942. (9) KING, C. G., H. W. KARN AND R. A. PATTON. Science **94:** 186, 1941. (10) KING, C. G., R. R. MUSULIN AND W. F. SWANSON. Am. J. Pub. Health **30:** 1068, 1940. (11) LEPKOVSKY, S., M. E. KRAUSE AND M. K. DIMICK. Science **95:** 331, 1942. (12) LINSTEAD, R. P. AND O. KRAYER. Science **95:** 332,

1942. (13) LUND, C. C. AND J. H. CRANDON. J. A. M. A. **116:** 663, 1941. (14) MAIER, N. R. F. AND N. M. GLASSER. Comp. Psych. Monog. **16:** 1, 1940. (15) MORGAN, C. T. AND H. WALDMAN. J. Comp. Psych. **31:** 1, 1941. (16) PATTON, R. A. J. Comp. Psych. **31:** 215, 1941. (17) PATTON, R. A., H. W. KARN AND C. G. KING. J. Comp. Psych. **32:** 543, 1941. (18) SEALOCK, R. R. AND H. E. SILBERSTEIN. Science **90:** 517, 1939. (19) SHWACHMAN, H. J. Ped. **19:** 38, 1941. (20) SIGAL, A. AND C. G. KING. J. Biol. Chem. **116:** 489, 1936; C. M. LYMAN AND C. G. KING. J. Pharm. and Exper. Therap. **56:** 209, 1936. (21) URBAN, F. AND H. B. PEUGNET. Am. J. Physiol. **123:** 207, 1938. (22) WILLIAMS, R. D., H. L. MASON, R. M. WILDER AND B. F. SMITH. Arch. Int. Med. **66:** 785, 1940; R. M. WILDER. War Medicine **1:** 143, 1941. (23) WILLIAMS, R. R. AND T. SPIES. Vitamin B_1 (Thiamin) and its use in medicine. Macmillan, 1938.

FOODS AS SOURCES OF THE VITAMINS

FRANKLIN C. BING

Department of Physiology and Pharmacology, Northwestern University Medical School, and the Office of the Council on Foods and Nutrition, American Medical Association, Chicago

Evidence has been accumulating in recent years to show that any complacency about the adequacy of the American diet is entirely unfounded. Diagnoses of pellagra, beriberi, scurvy and rickets appear in official mortality tables. The incidence of malnutrition in patients admitted to hospitals is great enough to have led some of our outstanding clinicians to make a special study of these conditions, and they have not lacked for material. Investigators are beginning to detect hitherto unrecognized signs and symptoms of vitamin A deficiency and of riboflavin deficiency in apparently healthy persons. In any group examined for the ascorbic acid content of the blood a surprisingly large number show low values which, while they do not indicate scurvy, doubtless indicate the desirability for dietary readjustment. The available evidence on the incidence of deficiency diseases recently has been surveyed and evaluated by a special committee of the Food and Nutrition Board of the National Research Council, composed of Drs. Norman Jolliffe, J. S. McLester, and H. C. Sherman. Their report (18) concludes with these significant words, "The evidence at our disposal warrants the conclusion that dietary inadequacies and malnutrition of varying degrees are of frequent occurrence in the United States and that the nutritional status of an appreciable part of the population can be distinctly improved. If optimal nutrition is sought, not mere adequacy, then widespread improvement is possible."

It has been emphasized by all groups who have studied the problem that general improvement in the nutritional status of the people can only come about through the wiser use of foods. Perhaps the most compelling reason for this conclusion is that we do not yet know as much about the dietary requirements as we should like and we do not yet understand the significance of some of the substances which are found in foods. Safety from a nutritional point of view is best assured by making wise selections of a variety of natural foods. It must not be forgotten moreover that foods provide iron and calcium and other important mineral salts, protein, fat, carbohydrate, and calories which are just as important as the vitamins which are the subject of the present discussion. There is at present a tremendous demand for information about foods and nutrition and there are many books and articles on these subjects. The present discussion is limited to the subject of vitamins in foods. It is not intended to be exhaustive, but to present certain viewpoints, and especially to point out the limitations of our knowledge, to emphasize the desirability of having more information about the composition of foods and the effects of processing on these values, and to point out also the desirability of all persons concerned in any capacity with the handling of foods learning something about the nutritional point of view.

VITAMIN REQUIREMENTS OF MAN. The vitamin requirements of the human being have been studied recently by scientific groups who were well qualified to express an opinion on the subject. In table 1 there are presented the minimum vitamin requirements as developed by the Food and Drug Administration for purposes of labeling products which are subject to the requirements of a section of the Federal Food Drug and Cosmetic Act which applies to foods which are represented to be, or purported to be, for special dietary purposes. In table 2 there are presented the recommended daily allowances for each of the important vitamins as developed by a committee of the National Research Council.

In considering these standards it should be men-

tioned that some of them are on a much firmer basis than are others. The minimum values represent a moderate level below which it would not be well to allow the diet to go. The underlying principle in formulating these minimum values was to select figures which were reasonably above the amounts on which symptoms of dietary deficiency might develop in the human being. The recommended daily allowances were intended to be more liberal. As pointed out by the committee which formulated these requirements, after consultation with a considerable number of persons interested in nutrition, the vitamin A requirements may be lower than the amount stated if the vitamin A is obtained primarily from animal sources such as milk and milk products and certain meat foods. If obtained largely from vegetable sources, perhaps even greater amounts would be desirable because carotene is not readily absorbed from the plant cells in which it occurs. The requirements for thiamine certainly are not as precisely known as might be indicated by such figures as 1.8 mgm. for a moderately active man weighing 153 pounds eating 3000 calories, 1.5 mgm. for a moderately active woman weighing 123.2 pounds and eating 2500 calories, 2.0 mgm. for a boy between the ages of 16 and 20 years and consuming 3800 calories, and so on through the list. It was intended by the committee selecting these values to indicate the nature in which thiamine requirements vary with activity, caloric intake (especially of carbohydrate foods) and age and sex. The requirements for adolescent boys are exceptionally high, higher than they are for any other age or sex group with the exception of the lactating mother. Few studies have been made of the riboflavin requirements, and practically nothing is known about the nicotinic acid requirements, but the committee formulating this table of recommended allowances believed that it would be desirable to show that these also probably vary with other physiologic factors much as the requirements for thiamine. The values for the requirements of ascorbic acid frankly are more or less of a compromise; some investigators believe they are higher, others are convinced they may be lower. In the case of vitamin D, a factor for which there is probably more evidence available than there is for the requirements of any other vitamin, there was a divergence of viewpoints so that a range of values has been selected.

In other discussions of this subject Dr. W. H. Sebrell has pointed out that if more were known about the actual vitamin requirements there would be no difference between minimum and optimal requirements, because the optimal presumably is the value which will permit no known pathology to develop and which will maintain the stores of the body at a maximum. Anything less than that could be considered insufficient.

The committee of the National Research Council also gave consideration to the requirements for vitamin K and decided that no decision need be made. The administration of vitamin K to pregnant women and to new-born babies is a well established therapeutic procedure and it was the opinion of the committee that it would be desirable to leave this matter in the hands of the obstetricians. The committee also considered that present knowledge did not warrant the making of any pronouncements about the requirements of other vitamins such as vitamin E and

TABLE 1

Minimum vitamin requirements

(Based on regulations of the Food and Drug Administration)

	VITAMIN A	THIAMINE	ASCORBIC ACID	RIBOFLAVIN	NICOTINIC ACID
	I.U.	*mgm.*	*mgm.*	*mgm.*	*mgm.*
Adult	4000	1.00	30	2.0	10*
Child (1 to 12 yrs. old)	3000	0.50-0.75	20		
Infant	1500	0.25	10	0.5	

* This value is not in regulations but has been recommended by The Food and Nutrition Board of the National Research Council. Vitamin D requirement is considered to be 400 I.U. or U.S.P. units irrespective of age.

TABLE 2

Recommended daily allowances for specific nutrients

(Food and Nutrition Board, National Research Council)

	VITAMIN A	THIAMINE	ASCORBIC ACID	RIBOFLAVIN	NICOTINIC ACID	VITAMIN D
	I.U.	*mg.*	*mg.*	*mg.*	*mg.*	*I.U.*
Man (154 pounds):						
Moderately active	5,000	1.8	75	2.7	18	
Very active		2.3		3.3	23	
Sedentary		1.5		2.2	15	
Woman (123.2 pounds):						
Moderately active	5,000	1.5	70	2.2	15	
Very active		1.8		2.7	18	
Sedentary		1.2		1.8	12	
Pregnancy (latter half)	6,000	1.8	100	2.5	18	400-800
Lactation	8,000	2.3	150	3.0	23	400-800
Children up to 12 years:						
Under 1 year	1,500	0.4	30	0.6	4	400-800
1-3 years	2,000	0.6	35	0.9	6	
4-6 years	2,500	0.8	50	1.2	8	
7-9 years	3,500	1.0	60	1.5	10	
10-12 years	4,500	1.2	75	1.8	12	
Children over 12 years:						
Girls: 13-15 years	5,000	1.4	80	2.0	14	
16-20 years	5,000	1.2	80	1.8	12	
Boys: 13-15 years	5,000	1.6	90	2.4	16	
16-20 years	6,000	2.0	100	3.0	20	

various members of the vitamin B complex that more recently are coming into prominence.

These tables of dietary allowances are useful only to those trained persons who recognize their limitations. The potentialities for misuse of the "dietary yardstick" are so great that Dr. Lydia J. Roberts, Chairman of the committee which drew up these values, repeatedly has emphasized the necessity of proper interpretation of the values in the table. The values have been translated into terms of foods by the Bureau of Home Economics (6), whose dietary patterns deserve the consideration of all persons who are likely to be asked for an opinion about the selection of foods for the maintenance of health.

DIETARY PATTERNS. It has been emphasized many times that the usual recommended pattern for an adequate diet will enable one to meet the requirements for each of the important vitamins with the exception of vitamin D. Because the securing of vitamin D represents a special problem for particular groups it can be met best either by the administration of pharmaceutical preparations or by the consumption of vitamin D milk, or even by the use of ultra violet irradiation. Suggestions about dietary patterns vary somewhat depending upon who is giving them and the purposes for which they are intended. In a chart entitled "A Guide to Good Eating" which has been prepared by the National Dairy Council and which has been considered and accepted by the Council on Foods and Nutrition of the American Medical Association the following list is provided:

Milk: 2 or more glasses daily—for adults
3 to 4 or more glasses daily—for children
To drink and combined with other foods

Vegetables: 2 or more servings daily besides potato—1 raw; green and yellow often

Fruits: 2 or more servings daily
1 citrus fruit or tomato

Eggs: 3 to 5 a week; 1 daily preferred

Meat, cheese, fish, or legumes: 1 or more servings daily

Cereal or bread: Most of it whole grain or "enriched"

Butter: 2 or more tablespoons daily

This pattern is very similar to others that have been offered during the last fifteen years. Briefly it may be noted that the plan emphasizes the important protective foods, milk and the green leafy and yellow vegetables. To these may be added as important classes of foods fruits, eggs, meat and other protein foods, and the whole grain or nutritionally improved cereal foods. About the only criticism that may be made of this list is that fortified oleomargarine is not mentioned as a possible substitute for butter. For low cost diets it has been necessary to stress the cereal foods and to decrease the eggs and meats to a recommended four servings weekly for the adult and to rely more on beans and other legumes for protein foods.

The foods in the quantities stated in the foregoing pattern provide about 1500 calories, which harmonizes with the view that one could well select about half his calories in the form of protective foods. It is obvious that some of the products in these different classes of foods have more food value than others. Dr. William B. Bradley and I have selected a number of common foods and have placed the values in a list and then have chosen the poorest and the best combinations according to the directions provided in the pattern. For this purpose we have made use of well known compilations of data on food composition plus the recently reported values for the nicotinic acid content of foods by Teply, Strong and Elvehjem (36). We have found that by making appropriate selections and still following this guide we could get diets that provided from 6,700 to 17,000 international units of vitamin A, from 1.1 to 2.9 mgm. of thiamine, from 1.8 to 5.4 mgm. of riboflavin, from 7.1 to 30 mgm. of niacin, and more than 100 mgm. of ascorbic acid regardless of the selection. The calculations show that this plan adequately provides for the vitamin A and the vitamin C requirements. In the case of the members of the vitamin B complex, however, the thiamine, riboflabin, and nicotinic acid content can be less than the recommended daily allowances, and indeed the riboflavin and nicotinic acid content can be less than the minimum requirement. To be certain of securing sufficient thiamine it is necessary to put more stress on whole grain or enriched cereal foods and on meats such as pork which are rich in this factor. To obtain sufficient riboflavin more emphasis must be placed on milk and perhaps on some of the meats. To obtain sufficient amounts of nicotinic acid it is necessary to select the whole grain or enriched cereals and the red meats. By substituting other foods for those mentioned one obtains low values such as we have calculated for the three important members of the vitamin B complex. It is evident therefore that dietary recommendations need to be more specific in order to make them mean what they are intended to be, that is, a model of a diet that is adequate for health. It is encouraging to know that the defects in the usually recommended dietary pattern, which have become recognized only as newer and more accurate data on the composition of foods have become available, are being studied by nutritionists with the view of correcting them.

As a nation we do not follow these guides to good eating, and indeed at the present time it would not be possible for us suddenly to adopt

such a plan. To provide every adult with a pint of milk daily and every child with a pint and a half to a quart or perhaps even more milk would mean that the milk production of the United States would have to be increased by 50 per cent. There would have to be an increase of from 50 to 100 per cent in the consumption of fruits rich in vitamin C and in the green, leafy vegetables. The meat consumption actually averages almost twice the recommended amounts in this plan; but, except for calcium and vitamin A, meats are excellent sources of the very factors in which our diets are apt to be deficient. Perhaps our present dietary habits are not so far wrong after all, because Stiebeling (35) recently has summarized her calculations of the nutritional value of the foods actually delivered to the kitchens of the nation and has concluded that the statistical average shows all values to be equal to the recommended daily allowances except for thiamine and riboflavin and calcium. Some of the other values are not as high as one would like to have them when it is recalled that the values are for the total food, and do not allow for waste or losses in cooking. Our American agriculture no doubt is capable of adapting its production to meet changes in demands for foods. Before any radical changes in agriculture are attempted, however, there ought to be evidence that such changes are in the interests of the improved nutrition of the general public. Conversely, the scientific aspects of any nutrition program should be correlated closely with the other fields of human endeavor that are involved in the feeding of the nation.

The late Dr. Mary Swartz Rose used to teach that every food ought to be given separate consideration, that each contributed certain substances to the dietary needs of man, and that with a knowledge of what a reasonably large number of common foods would supply one could then plan numerous diets that would adequately supply the dietary essentials. It is detailed dietetic information that the public needs today. The ordinary person should be provided with sufficient information to make it difficult for him or her to select anything less than an adequate diet.

VITAMIN CONTENT OF FOODS. *Difficulties of determination.* No one who has been concerned with any aspect of nutrition has progressed very far without being confronted with the need for accurate values for the composition of foods. We should know not only the average values but also the ranges of values that may be expected to be encountered (29). It is unnecessary to state here that the accurate estimation of the small amounts of vitamins in foods is not easy. The standard assay methods are tedious, time-consuming and expensive; it is difficult to preserve specimens of many kinds of foods properly for the duration of the assay. Although the chemical, physical and microbiological methods are invaluable and capable of solving problems that cannot be undertaken by the standard assay methods, yet the results must be received with caution until it has been demonstrated for each type of food investigated that the newer and older methods give comparable values.

Walker and Nelson (41) several years ago found by animal assay that the thiamine content of fresh yeast was apparently doubled if the yeast were dried before assay; presumably, the animal organism is not able to break down the live yeast cells efficiently to obtain the vitamin. With fresh yeast the shorter methods of assay give erroneously high results. Consider some of the difficulties that have developed in the cereal field —with wheat alone—just because of dependence on the results of non-standard methods of vitamin determination. From laboratory examination of the pigments of wheat it was believed that this grain contained a significant quantity of carotene (provitamin A); assays show it to have no detectable quantity (11). Until the methods of extraction were modified the rapid methods for determination of riboflavin in flour gave erroneously high values. On the other hand the nicotinic acid content of wheat was believed to be much less than it later was found to be. Many of the difficulties encountered in past discussions about the nutritional improvement of white bread and flour can be correctly attributed to lack of complete and accurate information about the composition of these products.

From lack of knowledge students of nutrition in the past have recommended the consumption of dark breakfast cereals, only to learn that often the color was produced by toasting, not by the incorporation of the aleurone cells of the wheat grain, which are rich in thiamine, and the toasting had destroyed most of the vitamin B_1 originally present.

The determination of the vitamin values of foods is basic for all other nutrition work. It is unfortunate that not many investigators have been attracted to this work and not many journals appear to be interested in publishing papers on food values or on methods of determining vitamins in foods. In the introduction to the book, which incidentally contains no information on vitamins, entitled "The Chemical Composition of Foods" by R. A. McCance and E. M. Widdowson (26) the problem is stated in the following way: "It need hardly be said that research of this nature is technically exacting and at the same time extremely laborious. Almost every substance to be analysed brings up fresh problems, and the solution of these problems may take weeks or even months. It is work which appeals to relatively

few investigators, for the road is long and there is little prospect of making interesting discoveries by the way." It would seem that the members of the food industry would find it profitable to determine the food value including the vitamin content of their products from the time that the material is obtained through each step in the processing until the product is finally delivered to the consumer. It is gratifying to note that industrial sponsorship of nutrition work, which has been increasing during recent years, has recently led to the formation of at least one research foundation of great promise. The food industry has a unique opportunity for contributing to the vast stream of information that is necessary in order to improve the nutritional status of the American people.

Variations in natural foods. There is an old fallacy that an egg is an egg, that beans are beans, but scientifically we know there are sometimes tremendous differences in the composition of different varieties of foods. Not only does the variety influence the food value but the soil in which plant foods are grown, the feeds on which animals are fed, the degree of maturity of fruits and vegetables, all affect the values. It has already been pointed out that pork is much richer in thiamine than other meats. Salmon contains more niacin than cod muscle (40). The vitamin A content of milk varies with the season (10). The thiamine content of beans has been found to vary from 1.0 to 2.9 international units to the gram depending on variety. Green snap beans are richer in carotene than yellow wax beans (4). Hard wheat contains more thiamine than soft wheat (3). American cheese contains about 8 micrograms of riboflavin to the gram, whereas cream cheese contains about 1.4 micrograms to the gram (9). Carrots vary in color and carotene content, the palest having from 7 to 12 micrograms to the gram and the darkest from 100 to 170 micrograms to the gram (34).

The vitamin C content of green snap beans has been found to vary from 0.09 to 0.28 mgm. to the gram depending on variety (24). In 7 varieties of strawberries the vitamin C content has been found to vary from 0.36 to 0.65 mgm. to the gram (33). Thirty-one varieties of cabbage have been tested for their vitamin C content and it has been found to vary from 0.48 for Allhead select to 1.81 mgm. to the gram for Midseason Market (7). When Kirk and Tressler (20) developed their method for the determination of ascorbic acid in pigmented foods they found the ascorbic acid content of eleven varieties of strawberries to vary from 0.40 to 1.04 which agrees reasonably well with the figures already quoted. In seven varieties of raspberries the variation was from 0.13 to 0.30, for six varieties of blueberries from 0.13 to 0.20 and for five varieties of turnips from 0.32 to 0.47 mgm. to the gram. All of these foods are rich sources of ascorbic acid. Some, such as cabbage, contain much more than the citrus fruits.

Of course many more examples could be cited of variations in nutritional value of different varieties of plant foods, but there is a need for obtaining still more information along these lines. No doubt information of this nature will be made available by important research centers such as the U. S. Plant, Soil and Nutrition Laboratory that has been formed at Ithaca under Professor Maynard. Whether variations in nutritive value of different varieties of plants are dependent on genes or chromosome numbers or both is one of the factors that needs to be determined. It has been suggested that agricultural economists might well give consideration to the yield in nutritional value to the acre in addition to the other values which they use to measure crop production. Obviously the most desirable plant variety is that which yields the maximum of nutritional value, but of course consideration will have to be given to other factors, such as resistance of different varieties to plant diseases, quantitative yield, and the like. A line of food research of interest in this connection is that undertaken in the State of Maine on sources of ascorbic acid. There Murphy (30) has pointed out that many of the persons living in that state rely on home grown crops for their fruits and vegetables. Because of climatic conditions tomatoes are not grown to any appreciable extent. Apples are available but those varieties which have been selected happen to be those which are rather low in vitamin C content. One of the crops that seemed to offer possibilities as a practical source of vitamin C was onions. An investigation showed that sixteen varieties had values ranging from 0.17 to 0.40 mgm. of ascorbic acid to the gram but only ten of these varieties reached maturity under conditions of cultivation. For these, the values obtained were from 0.13 to 0.22 mgm. to the gram. Investigations of this nature are capable of yielding data of tremendous practical value in nutrition.

Effects of soil, season, maturity and storage on vitamin content. A few studies have been made of the effects of soil and season and more studies have been made on the vitamin content of food crops at different stages of maturity. Investigators at the New York Agricultural Experiment Station (38) have reported that spinach grown on muck soil had 0.49 mgm. of ascorbic acid to the gram, while that grown on upland soil had 0.75 mgm. to the gram. At this meeting a group of workers (16) have reported that the ascorbic acid of hothouse grown tomatoes is considerably less than that produced in tomatoes grown outdoors during the regular season. Investigations

such as these provide the kind of data people want to know.

The vitamin C content of bananas has been shown to be greater in the fully ripe banana than in the green or overripe banana (17, 21). Oranges picked while green, contrary to what might be supposed, do not have a lower content of vitamin C than they do when picked fully ripe; indeed they may have a somewhat higher content. The vitamin C content of immature peas is higher than it is in mature or over-mature peas (25). On the other hand the thiamine content is almost negligible in small green peas and is highest in the dried peas that have been allowed to grow to maturity. Dry peas contain no ascorbic acid unless they have been soaked in water and are beginning to sprout at which time they rate as rich sources of this dietary essential. The ascorbic acid content of green snap beans or spinach changes little during maturation.

During storage it is commonly thought that the vitamin content decreases. This is not necessarily true. With carrots, for example, stored under conditions of refrigeration, the color increases and the carotene content does also. Smith and Otis (34) in one experiment found that the value increased from 48 to 98 micrograms to the gram in two months and in another sample stored in a refrigerator at about 4°C. the carotene content increased from 66 to 102 micrograms to the gram between January and April. There is little loss in vitamin C of cabbage even at room temperature for moderate periods. At a temperature of 8 to 9°C. the ascorbic acid content was found to decrease from 0.32 to 0.22 in 56 days and to 0.20 mgm. per gram in 84 days (15). Stored at from 1 to 3°C. spinach loses vitamin C slowly, there being practically no change in a week. If stored at from 23 to 26°C., however, nearly all of the vitamin C is lost in one week and about one-half of it within three days. Similarly there is little loss of vitamin C of peas in the pod if stored at from 1 to 9°C. but considerable loss at temperatures from 18 to 22°C. (25).

The applications of observations such as these are obvious. Fresh vegetables which show loss of important nutrients at room temperatures could well be kept under conditions of refrigeration until used. In general the canner and other food processors could well afford to allow as little time as possible to elapse between harvesting and preserving the crops. However, this aspect of practical nutrition is very much an empirical science and each food deserves special study for each of the important dietary essentials, of which the vitamins are only one group.

Losses in processing. Commercial processing of foods includes refrigeration and quick freezing, drying and dehydration, and canning.

Frozen foods. The literature on the nutritive aspects of quick frozen foods has been reviewed by the late Mary Swartz Rose (32). She concluded that "good quality, freshness, sanitary care, subjection to low temperatures as speedily as possible, holding at −17.8°C. (0 F.) or lower while in storage, in an airtight waterproof package, and maintenance in the frozen condition until time of using are essential conditions for producing safe foods of the highest nutritive value." Under usual conditions the vitamin A and riboflavin values of foods seem to be well preserved in quick frozen foods. The thiamine may be considerably destroyed during blanching prior to freezing or in subsequent cooking of the foods. Vitamin C may be lost in blanching or in thawing of the frozen foods before consumption. For the latter reason it is frequently recommended that quick frozen foods be cooked without defrosting.

As an example of the type of contribution that can be made in this field mention may be made of the report of Tressler and his collaborators (39). They showed that decreasing the time of blanching of lima beans resulted in an appreciable reduction in the loss of ascorbic acid, while the processing still was sufficient to destroy the enzymes which are responsible for the development of off-flavors and loss of color in poorly processed beans.

Dried and dehydrated fruits and vegetables. Except for a few products sun dried and commercially dehydrated fruits and vegetables have been relatively minor items of production. There is intense interest in these foods at the present time however. The literature on dried druits has been reviewed by Agnes Fay Morgan (28) and on both fruits and vegetables by Tressler (37). Carotene and ascorbic acid may be destroyed by ordinary processing, and thiamine by exposure to sulfur dioxide, which on the other hand aids in the preservation of color and perhaps of carotene. Improvements in processing of prunes have resulted in the production of an exceptionally valuable food. More study is needed, however, of possibilities of conserving the food values of other foods during processing and storage. Plans for research in this field under government auspices already are well formulated.

Canned foods. More foods are canned than are preserved in any other way. The evidence shows that carotene and riboflavin in most vegetable foods are well retained during processing and subsequent storage. Some products such as the citrus fruit juices and tomato juice, can be canned without loss of ascorbic acid. Little is known about the preservation of ascorbic acid in other foods. Available data indicate that loss of thiamine may be considerable (1).

All too often the nutritional point of view has

been notoriously lacking among leaders in the commercial food field. How refreshing it is to read a technical paper on the canning of fruit juices such as that reported by Lueck and Pilcher (23). These investigators have provided an authoritative discussion of the problems of the commercial canning of fruit juices, including tomato juice. They compared the older steam jacketed kettle method of heat-processing of fruit juices with the newer so-called high-short process, wherein the juice is heated in a few seconds to temperatures which are sufficient to destroy both spoilage and pathogenic organisms which might multiply in the food at ordinary temperature. Florida grapefruit juice processed by the kettle heating method had, in the first juice drawn off, 0.29 mgm. of ascorbic acid per cubic centimeter, in the juice last drawn off, 0.06 mgm. of ascorbic acid to each cubic centimeter. The same juice when processed by the flash-pasteurization or high-short method contained 0.40 mgm. of ascorbic acid per cubic centimeter. Because of contact of the juice with air, there is marked destruction of ascorbic acid during the 15 to 30 minutes usually required to bring the juice to proper temperatures and during the additional 30 minutes or more which may be required to empty the kettle. These investigators also have discussed results of bio-assay and concurrent chemical titration for vitamin C of samples of tomato juice packed in different types of containers. In harmony with the results obtained by other investigators, they found that ascorbic acid is retained well during storage in tin cans, but poorly when stored in glass. The investigation of Lueck and Pilcher is a model of the type of study that should be made of other commercially canned fruit juices, as well as other commercially canned products.

That the high-short method of heat processing is worthy of further study from the nutritional point of view is indicated by some observations of Pilcher and Feaster (22). In an experimental pack of corn, the following results were obtained on the thiamine content expressed in terms of international units to each 100 grams.

Type of corn	*Process*	*Vitamin B_1 content I.U. per 100 grams*
White	Short-high	27.7
	Conventional	16.8
Yellow	Short-high	19.6
	Conventional	12.1

EFFECT OF COOKING ON THE VITAMIN VALUES OF FOODS. Except for vitamin C the development of improved methods for the determination of small amounts of vitamins in foods has been too recent to have permitted the accumulation of many data on the effect of home preparation of foods on their vitamin content. There have been a few excellent studies, however, which indicate the type of investigative work that is necessary. Aughey and Daniel (2) studied the loss of thiamine which resulted from cooking in a number of foods, the determinations being made by the bio-assay method. They found no loss of thiamine as a result of cooking carrots by boiling or in a pressure cooker. About a third of the thiamine content of potatoes was lost by boiling, less by baking. Raw spinach contained 43 I.U. of thiamine per 100 grams and boiled spinach 30 I.U. per 100 grams, corrected for loss of water during cooking. Green peas contained 150 I.U. per 100 grams when raw and, after simmering for 12 minutes, they contained 120. If a pinch of soda had been added, the peas were greener in color but had a little less thiamine, or 100 I.U. per 100 grams. Snap beans contained 22 I.U. per 100 grams when raw, 15 after boiling for 40 minutes, 9 after boiling for the same time with a little pinch of soda, these values again being calculated to the same moisture content. Navy beans lost no thiamine on soaking and cooking; the addition of a trace of soda reduced the cooking time from 83 to 53 minutes, with no loss of thiamine. Kelly and Porter (19) likewise found no loss of thiamine on boiling beans, with or without the addition of soda during the soaking process. The cooking of rolled oats or of whole wheat in a double boiler was attended by no loss of thiamine. A loss of 14 per cent of the thiamine occurred when wheat flour was baked in the form of bread. There was a loss of 15 per cent of the thiamine of pork as a result of braising, but 57 per cent loss from roasting. These results with meat correspond well with the observation of Mickelsen, Waisman, and Elvehjem (27). Aughey and Daniel called attention to the changes in water content as a result of cooking. For this reason in part a serving of baked potatoes provides more thiamine than a serving of cooked whole wheat or oats. Boiled potatoes, cooked spinach, and carrots each furnish amounts of thiamine per serving which are comparable to those supplied by cooked cereal or a slice of whole wheat bread.

A great deal of research has been done on the effects of different cooking procedures on the ascorbic acid content of foods (5, 8, 13, 14). Much of this has been reviewed by Fenton (12). One exceedingly practical study is that reported by Richardson, Davis and Mayfield (31) on the effects of cooking on the vitamin C content of potatoes. They report no loss on boiling potatoes for 35 minutes, and an apparent increase in steaming. A serving of potatoes, about 150 grams or one medium sized potato, will provide from 14 to 25 mgm. of ascorbic acid depending on whether the potatoes are American fried or baked. There

is evidence that the ascorbic acid is largely destroyed by mashing potatoes or by whipping.

Some day there will be a cook book that is really based on nutritional principles. Doubtless such a volume will dispel many of our present prejudices. Frying is considered to be the least desirable method of preparing foods and yet it has been found, as already stated, that fried meats retain more thiamine. The addition of bicarbonate of soda has been objected to on the grounds that it aids in the destruction of thiamine and of ascorbic acid, which is true in most products, but in the cooking of dry beans it has been shown that a trace of bicarbonate of soda has no deleterious effect and appreciably shortens the cooking time.

CONCLUSIONS. Foods supply all of the nutritional essentials required by man but it is necessary to make a wise selection of them in order to secure an adequate diet. There is need for a thorough investigation by the newer methods of the vitamin content of different varieties of foods with a view to selecting for crops those which, other matters being equal, yield the greatest nutritive value. There is need also for additional accurate studies of the vitamin content of foods at different stages of processing with a view to developing commercial methods of preservation that will retain to the highest degree possible the natural vitamin endowment of the foods. In the development of such investigations the food industries have a unique opportunity of contributing to the public welfare. Finally, there is a need for more information on the effects of cooking and home preparation of foods on the vitamin values. When such information is obtained in detail and has been suitably verified then it will be possible to develop satisfactory guides for the eating of foods and satisfactory nutritional programs for the nation.

REFERENCES

(1) ARNOLD, A. AND C. A. ELVEHJEM. Food Research **4:** 547, 1939. (2) AUGHEY, E. AND E. P. DANIEL. J. Nutrition **19:** 285, 1940. (3) BOOHER, L. E. AND E. R. HARTZLER. Tech. Bull. no. 707, U. S. Dept. Agriculture, Bureau of Home Economics, 1939. (4) BOOHER, L. E. AND R. L. MARSH. Tech. Bull. no. 802, U. S. Dept. Agriculture, Bureau of Home Economics, 1941. (5) BROWN, E. J., H. SCHUELE AND F. FENTON. Food Research **6:** 217, 1941. (6) Bureau of Home Economics, U. S. Dept. of Agriculture. Planning Diets by the New Yardstick of Nutrition, 1941. (7) BURRELL, R. C., H. D. BROWN AND V. R. ELRIGHT. Food Research **5:** 247, 1940. (8) CURRAN, K. M., D. K. TRESSLER AND C. G. KING. Food Research **2:** 549, 1937. (9) DAY, P. L. AND AND W. J. DARBY. Food Research **1:** 349, 1936. (10) DORNBUSH, A. C., W. H. PETERSON AND F. R. OLSON. J. A. M. A. **114:** 1748, 1940. (11) Editorial. Lack of Provitamin A in Flour. J. A. M. A. **115:** 1725, 1940. (12) FENTON, F. J. Am. Diet. Assoc. **16:** 524, 1940. (13) FENTON, F., D. K. TRESSLER AND C. G. KING. J. Nutrition **12:** 285, 1936. (14) FENTON, F., D. K. TRESSLER, S. C. CAMP AND C. G. KING. Food Research **3:** 403, 1938. (15) GOULD, S., D. K. TRESSLER AND C. G. KING. Food Research **1:** 427, 1936. (16) HAMNER, K. C., C. B. LYON, G. H. ELLIS AND K. C. BEESON. Fed. Proc. **1:** 189, 1942. (17) HARRIS, P. L. AND G. L. POLAND. Food Research **4:** 317, 1939. (18) JOLLIFFE, N., J. S. MCLESTER AND H. C. SHERMAN. J. A. M. A. **118:** 944, 1942. (19) KELLY, E. AND T. PORTER. Food Research **6:** 85, 1941. (20) KIRK, M. M. AND D. K. TRESSLER. Food Research **6:** 395, 1941. (21) LEVERTON, R. Food Research **2:** 59, 1937. (22) LUECK, R. H. Sheet Metal Industries **13:** 1243, 1345, 1939. (23) LUECK, R H. AND R. W. PILCHER. Indust. and Eng. Chem. **33:** 292, 1941. (24) MACK, G. L., W. T. TAPLEY AND C. G. KING. Food Research **4:** 309, 1939. (25) MACK, G. L., D. K. TRESSLER AND C. G. KING. Food Research **1:** 231, 1936. (26) MCCANCE, R. A. AND E. M. WIDDOWSON. Chemical Composition of Foods, Special Report no. 235, Medical Research Council, London. (27) MICKELSEN, O., H. A. WAISMAN AND C. A. ELVEHJEM. J. Nutrition **17:** 269, 1939. (28) MORGAN, A. F. Am. J. Public Health **25:** 328, 1935. (29) MUNSELL, H. E. J. Am. Diet. Assoc. **17:** 311, 1941. (30) MURPHY, E. F. Food Research **6:** 581, 1941. (31) RICHARDSON, J. E., R. DAVIS AND H. L. MAYFIELD. Food Research **2:** 85, 1937. (32) ROSE, M. S. J. A. M. A. **114:** 1356, 1940. (33) SATTERFIELD, G. H. AND M. YARBROUGH. Food Research **5:** 241, 1940. (34) SMITH, M. C. AND L. OTIS. Food Research **6:** 143, 1941. (35) STIEBELING, H. K. J. A. M. A. In press, 1942. (36) TEPLY, L. J., F. M. STRONG AND C. A. ELVEHJEM. J. Nutrition **23:** 417, 1942. (37) TRESSLER, D. K. In press, 1942. (38) TRESSLER, D. K., G. L. MACK AND C. G. KING. Food Research **1:** 3, 1936. (39) TRESSLER, D. K., G. L. MACK, R. R. JENKINS AND C. G. KING. Food Research **2:** 175, 1937. (40) WAISMAN, H. A. AND C. A. ELVEHJEM. The vitamin content of meat. Burgess Publishing Co., Minneapolis, 1941. (41) WALKER, R. AND E. M. NELSON. Am. J. Physiol. **103:** 25, 1933.

THE BIOLOGICAL ACTION OF THE VITAMINS

C. A. ELVEHJEM

Department of Biochemistry, University of Wisconsin, Madison

When I discuss the biological action of vitamins with various individuals I generally encounter two different reactions. Some are surprised at the lack of knowledge concerning the function of vitamins in metabolism and others are amazed at the progress that has been made in this field during the past few years. I need only to remind those of the first group that our knowledge of the biological action of glucose, of fatty acids and of amino acids is still far from complete. In fact studies on the function of the vitamins are so intimately related to the metabolism of the above compounds that any progress in one field is of distinct value in the other. However, I, too, wonder sometimes how long it will take to get some idea regarding the specific function of vitamin A in the maintenance of epithelial tissues or of vitamin D in bone formation. Again it is possible to understand the reaction of the second group if we limit our consideration to a few vitamins such as thiamin, riboflavin, and nicotinic acid but when we get beyond this group we have nothing to be enthusiastic about.

The pioneer workers in the field of vitamins developed their theories regarding the function of vitamins around the symptoms observed in their deficient animals. It was natural for anyone working during that period, i.e., the close of the last century, to ascribe these symptoms to the presence of pathogenic organisms, since the work of Pasteur and others seemed to indicate that every disease must have a positive causative agent. Eijkman (1) in 1897 received little support when he suggested that beri-beri was not due to the presence of pathogenic organisms in rice but to a state of intoxication brought about by the metabolism of excessive quantities of starch. In 1907 Hart and co-workers (2) found that calves fed diets compounded from the wheat plant failed while those on similar diets made from the corn plant survived in good health. At that time they postulated an inherent toxicity in the wheat grain, but 17 years later they (3) were able to show conclusively that these early results were due to a vitamin A deficiency brought about by the very low amount of vitamin A in wheat grain. Many of you remember the opposition to Goldberger's (4) conclusion that pellagra was caused by a lack of a vitamin rather than the consumption of moldy corn.

More recently we have seen a similar story in the case of egg white toxicity and biotin. Practically all workers who have studied the syndrome since it was first described by Boas (5) in 1927 have referred to it as some form of toxicity. Parsons and co-workers (6) concluded that the dermatitis in rats fed egg white was dependent upon an interrelation between a positive toxicity and a relative absence of a protective factor. Today due to the work of György, Rose, Eakin, Snell and Williams (7) we know that the egg white combines with biotin in the intestinal tract and that the syndrome of egg white toxicity is actually due to a biotin deficiency.

Histological study of the tissues from vitamin deficient animals constitutes another approach toward the elucidation of the mechanism of vitamin action. A large body of literature has accumulated on this subject and much is now known concerning the effect of various vitamin deficiencies upon the intimate structures of certain tissues. However valuable such studies may be, it is apparent that the histological observations are to a great extent descriptive and the fundamental change underlying these structural changes will be elucidated only when our knowledge of the metabolic disturbances is more complete.

A possible functional relationship between vitamins and hormones has been offered as an attractive hypothesis for the explanation of vitamin action. Unfortunately our information on this subject is far too meager to allow any establishment of such relationship. Within the past few months McKibbin in our laboratory has found that cortical extract given to dogs with severe pantothenic acid deficiency will prolong their lives for several weeks. Daft, Sebrell, Babcock, and Jukes (8) as well as others have described adrenal damage in rats deficient in pantothenic acid but in spite of these facts it is impossible to conclude that the function of this vitamin is related directly to the cortical hormone.

During the past decade a greater emphasis has been placed on the relation of enzymes to vitamin deficiencies. This change is partly due to an increasing knowledge of the chemistry of enzymes and partly to the availability of crystalline vitamins, a condition which has not only aided in the vitamin studies but in many of the fundamental investigations on enzymes themselves.

In 1921 Seidell (9) stated that aside from a possibly significant degree of dialyzability there is no outstanding evidence that vitamins should not be classified with enzymes. Today we know that the difference in dialyzability is an important distinction between these two groups of compounds. By 1931 Neuberg and Euler had proposed the term cozymase for the coenzyme of

alcoholic fermentation and apozymase for the enzyme free from the coenzyme. I believe that everyone today agrees that the major part of an enzyme is protein in nature and that under most conditions the living cell has little difficulty in synthesizing these proteins. We may find that a greater part of the total protein in the body is present in the form of enzymes than most workers suspect. The activity of these protein catalysts is greatly altered by the presence of activators. These specific activators may be divided into kinases, which are generally colloidal in nature, and coenzymes, which are crystalloidal. Apparently the living organism has greatest difficulty in producing the structures that go to make up coenzymes and according to our present knowledge at least some of the vitamins are directly concerned with these coenzymes. More relationships will undoubtedly be established upon further work, but we must not expect to find that the function of all the vitamins is related to enzymes.

Let us consider thiamin first. In any discussion of the biological action of thiamin we immediately turn to the classical observation of Lohmann and Schuster (10) in 1937. These workers isolated cocarboxylase in crystalline form and showed it to be the pyrophosphoric acid ester of thiamin. This observation recalls the early work of Neuberg and Hildescheimer (11) who reported the presence in yeast of an enzyme called carboxylase which catalyzed the decarboxylation of α-keto acids. In 1932 Auhagen (12) split this enzyme into a protein component and a thermostable factor, cocarboxylase. Within the past year Green, Herbert and Subrahmanyan (13) isolated carboxylase from top brewer's yeast and found it to be diphosphothiamin magnesium protein. The best preparation contained 0.46 per cent diphosphothiamin and 0.13 per cent magnesium.

All this work was done with yeast but work carried out simultaneously in Peters laboratory (14), as well as in our laboratory (15), had shown that a decreased respiration occurred in tissues from polyneuritic animals when pyruvate was used as a substrate and that the addition of thiamin stimulated both oxygen uptake and pyruvate removal. Numerous in vivo studies have verified the conclusion that thiamin is related to pyruvate metabolism. It is fairly simple to demonstrate a reduced cocarboxylase content in tissues from experimental animals deficient in vitamin B_1. Ochoa and Peters (16) report about 3 micrograms of cocarboxylase per gram of normal brain tissue in rats and this value decreases to less than one microgram during avitaminosis. Similarly the liver tissue showed a decrease from about 5 micrograms to less than one microgram after the development of polyneuritis. Goodhart and Sinclair (17) found a significant decrease in the blood cocarboxylase during human avitaminosis. It is interesting that Simola (18) in 1932 observed that the cocarboxylase content of tissues from rats suffering from complicated vitamin B deficiency was lowered significantly but little attention was given to this report until Lohmann and Schuster's work appeared.

There is ample evidence that cocarboxylase is the active form of thiamin in the animal body and that thiamin is phosphorylated by means of the phosphate-transferring reaction of the Embden-Meyerhof scheme. However, the reactions in which cocarboxylase is concerned are much more complex in animal tissues than in yeast where pyruvic acid is decarboxylated to acetaldehyde and CO_2. Green, Westerfeld, Vennesland and Knox (19) have prepared a crude preparation of the carboxylase from animal tissues but the protein is undoubtedly different from that found in yeast. Barron et al. (20) reported that cocarboxylase accelerates the following reactions in animal tissues: synthesis of carbohydrate, synthesis of α-ketoglutarate, synthesis of citrate, synthesis of succinate and synthesis of acetoacetate. All these reactions start as condensation products of pyruvate and contain a step involving oxidative decarboxylation.

The action of thiamine has also been related to acetyl choline but the direct relationship is not clear. Mann and Quastel (21) have demonstrated that the rate of synthesis of acetyl choline with pyruvate as substrate is greatly diminished in polyneuritic brain tissue. The addition of thiamine to the polyneuritic brain restored the synthesis to normal. An inhibiting action of thiamine upon choline esterase has also been observed (Bernheim (22)). Thus it would appear as though thiamin deficiency would result in an increased choline esterase activity which in turn would lead to a lower acetyl choline concentration.

Riboflavin is related directly to more enzymes than any of the known vitamins. It is well known that Warburg and Christian (23) isolated from yeast in 1932 a flavoprotein which was considered as a carrier link between coenzyme II and molecular oxygen. This flavoprotein was resolved in acid solution into a protein and riboflavin phosphate. The enzyme was reduced by reduced coenzyme II and reoxidized by molecular oxygen. During the early studies these reactions were carried out in flasks containing pure oxygen. This system would have very little activity in animal tissues where the oxygen tension is at a low level. Since that time several different flavoproteins related to the pyridine coenzymes have been studied.

In 1938 Haas (24) described the isolation of a flavoprotein from yeast which catalyzed the re-

action between coenzyme II and methylene blue. In contrast to the original compound of Warburg and Christian, this enzyme contained alloxazine-adenine dinucleotide rather than riboflavin phosphate as the prosthetic group. At about the same time Dewan and Green (25) and Euler (26) prepared concentrates of an enzyme which catalyzed the transfer of hydrogen from coenzyme I to methylene blue. Later this enzyme was isolated from heart muscle by Straub (27) and was found to contain the dinucleotide. None of these compounds, however, catalyzed the reaction between the coenzymes and cytochrome which is the most important pathway in animal tissues.

Very recently Haas, Horecker and Hogness (28) isolated a flavoprotein which does catalyze the reaction between coenzyme II and cytochrome C. It is interesting that this enzyme contains the flavin phosphate found in the original yellow enzyme rather than the dinucleotide. Still more recently Altschul, Persky and Hogness (29) have isolated a soluble enzyme which catalyzes the reaction between coenzyme I and cytochrome. At present the constitution of this enzyme has not been determined. This work indicates the significance of flavoproteins in respiratory mechanisms, but we must emphasize that both of these compounds have been isolated from yeast and not from animal tissues. As far as I know, no one has measured the amount of these flavoproteins in tissues taken from animals suffering from riboflavin deficiency. However, there are other flavoproteins which catalyze the direct reaction between the substrate and molecular oxygen and may therefore be classed as dehydrogenases. One of these is d-amino acid oxidase which catalyzes the oxidative deamination of several unnatural amino acids and which, according to Warburg and Christian (30) contains the dinucleotide as the prosthetic group. Two years ago we (31) demonstrated in our laboratory that a riboflavin deficiency in rats resulted in a lowered d-amino acid oxidase content of the liver and kidney. Ochoa and Rossiter (32) demonstrated similar decreases in the total riboflavin dinucleotide content of heart and liver from rats showing riboflavin avitaminosis. The dinucleotide content is rapidly restored to its normal level by the administration of riboflavin to the deficient rat. This indicates the ability of the living tissue to synthesize the nucleotide when sufficient riboflavin is supplied. Klein and Kohn (33) have demonstrated this synthesis by human blood cells both in vitro and in vivo.

Another flavoprotein belonging to this group is xanthine oxidase. Ball (32) and Corran and coworkers (35) have shown that the prosthetic group of this enzyme consists, at least in part, of the dinucleotide. We (36) have recently shown that the xanthine oxidase activity of rat liver is greatly reduced in riboflavin deficiency. The xanthine oxidase activity is rapidly restored to normal when riboflavin is fed and the rate of growth restricted but with rapid growth the restoration is somewhat delayed. In this case some evidence was obtained that the protein component of the xanthine oxidase was also diminished during the deficiency. Thus in the absence of the prosthetic group less of the protein is manufactured.

Very recently we have demonstrated a rather definite decrease in the succinic acid oxidase content of the liver and heart from riboflavin deficient rats. Up to the present time no one has shown conclusively that succinic acid oxidase contains riboflavin, but the above results may indicate that such is the case. It is impossible to correlate the changes in the individual enzymes with the variety of symptoms that have been associated with riboflavin deficiency in experimental animals and humans, but from the short survey given it is evident that riboflavin plays an important role in the entire respiratory mechanism in the animal body. The recent report by Sure and Dichek (37) that riboflavin produces a profound effect on the economy of food utilization for syntheses of body tissues is further evidence that metabolism proceeds more efficiently when there is sufficient riboflavin for the optimum activity of the various enzyme systems.

According to our present knowledge, nicotinic acid is related to only two coenzymes with very similar composition, but the entire picture is quite complicated because these coenzymes enter into so many different reactions. Harden and Young first described a coenzyme for yeast fermentation. In 1918 Meyerhof found this coenzyme in a number of animal tissues. The necessity of a coenzyme for the oxidation of lactic acid by lactic acid dehydrogenase of animal tissues was demonstrated by Szent-Györgyi in 1925.

Now if we return to the early work of Warburg and Christian on flavoproteins we find that these workers used in their enzyme system a coenzyme II. In 1935 it was isolated and characterized as a dinucleotide consisting of adenine, pentose, phosphoric acid, and nicotinic acid amide. Euler and his coworkers had been working on cozymase or coenzyme I from yeast and in the same year both Euler and Warburg isolated nicotinic acid amide from the cozymase after hydrolysis. The two compounds differ only in the number of phosphoric acid molecules but show great specificity as far as the protein or dehydrogenase with which they may function. The two coenzymes show about the same affinity for their specific proteins but the affinity is much less than that for the rigidly bound flavoproteins.

Although nicotinic acid, adenine, and ribose are constituent parts, nicotinic acid appears to be the only compound which the animal body has difficulty in making. Even the nicotinic acid can be produced by animals such as the rat and chick. It is, of course, well known that certain microorganisms such as the *Hemophilus para-influenzae* will grow only in the presence of the coenzyme and will not respond to the free acid or amide (38). However, this fact is probably not due to the organism's inability to synthesize the ribose or the adenine. We have tried a number of times to demonstrate the essential nature of adenine or adenylic acid in animal nutrition with no success. However, this does not prove that the animal body can synthesize adenylic acid in adequate amounts because the basal ration may still carry considerable quantities. Spies has reported beneficial effects in humans with adenylic acid but again this does not indicate that the normal individual cannot make it in adequate quantities. Very recently we have tried to demonstrate the essential nature of ribose in the diet of rats receiving limiting amounts of riboflavin but again no very significant differences have been observed between the rats with and without added ribose.

Some of the most interesting observations on the production of cozymase come from studies involving sulfapyridine. West and Coburn (39) found that sulfapyridine prevented the growth-stimulating effect of nicotinic acid on *Staphylococcus aureus* grown on a medium deficient in nicotinic acid. West (40) extended this study to dogs and found that if a black tongue dog is saturated with sulfapyridine the dog will no longer respond to nicotinic acid therapy. However, raw liver was found to be highly efficacious in curing the black tongue. West concluded that the fresh liver supplied preformed coenzymes but it is difficult to accept the suggestion that coenzymes will go through the digestive tract and be absorbed without degradation. We have repeated West's experimental procedure with identical results but we are more inclined to believe that the fresh liver supplies a factor affecting cozymase formation.

It is apparent then that the most important limiting factor for the production of coenzymes under normal conditions is nicotinic acid. Numerous studies have been made on the changes in the coenzyme content of tissues from animals and humans during nicotinic acid deficiency.

It is impossible to discuss here the methods involved in these measurements but in general two types of procedure have been used. One is based on the growth responses obtained with *H. parainfluenzae* and measures both coenzyme I and II as well as possible other factors, and the other one is based on the specific reactions catalyzed by coenzyme I during yeast fermentation. Axelrod in our laboratory has used the original fermentation test for cozymase with considerable success. Jandorf, Klemperer and Hastings (41) have recently described a method which involves only part of the fermentation mechanism. In both cases CO_2 evolution is measured although in the fermentation method the CO_2 is produced from the entire reaction while in the latter the CO_2 is evolved from a sodium bicarbonate buffer by the acid produced.

All methods depend upon the use of a standard preparation of cozymase. Since pure cozymase is difficult to obtain and definite criteria for its purity are lacking, considerable controversy has arisen concerning the true cozymase value of animal tissues. We (42) have consistently found about 1,000γ of cozymase per gram of fresh liver and kidney tissue and about 600γ for muscle tissue. Handler and Dann (43) report much lower values and criticize our results on the basis of improper evaluation of the purity of our standard nucleotide. We must admit that our standard may not represent 100 per cent cozymase and therefore our values may be a little high, but this does not explain all the discrepancies. Cozymase is a very labile factor in the presence of other tissue enzymes and the tissues must therefore be handled with great care to prevent decomposition of the coenzyme. The low amount of cozymase and the high amount of nicotinic acid in liver tissue reported by Handler and Dann (43) may be due to decomposition of the coenzyme during preparation of the tissues for analysis. Recognition of this fact is even more important in light of the work by Mann and Quastel (44) which shows that a cozymase nucleotidase occurs in rat brain and liver but is present to a relatively small extent in rat kidney. The activity of cozymase is destroyed by this nucleotidase but the presence of nicotinic acid amide retards the inactivation. The importance of this nucleotidase in the intact tissues is unknown.

In spite of the discrepancies in the absolute amount of cozymase in various tissues, all workers are agreed that the cozymase content of the liver and muscle tissue decreases during nicotinic acid deficiency. The values reported by Kohn, Klein and Dann (45) and Axelrod, Madden and Elvehjem (46) indicate a reduction of about 70 per cent in the liver and 35 per cent in the muscle in the case of the dog. Axelrod, Spies, and Elvehjem (47) found the coenzyme I content of the muscle of pellagrins to decrease as the deficiency increased in severity. In all cases the coenzyme content increased with therapy. Most workers, I believe, now agree that the blood cozymase content undergoes little change during nicotinic

acid deficiency. Typical changes may range from 85γ per ml. in normal blood to 69γ per ml. in the blood from severe pellagrins. It is interesting in this connection that Kohn and Klein (48) have demonstrated the in vitro synthesis of cozymase from nicotinic acid in red blood cells. Although no specific measurements have been made on coenzyme II during nicotinic acid deficiency, I believe we are safe in concluding that the function of nicotinic acid in the animal body is largely related to these two coenzymes. Much more work is necessary before we can relate the observed change in coenzyme content of certain tissues to the external symptoms observed.

The remaining members of the vitamin B complex have not been associated with any specific enzymes, although there are reasons to suspect certain relationships. It is well known that pyridoxine, pantothenic acid, and biotin are closely associated with proteins in the living cell. One may immediately suggest that we now have a pretty complete knowledge of the various components of the respiratory enzyme system and there is no place for these compounds. That is true to some extent but there are still many gaps. There is an unknown component in cytochrome C which, by the way, contains sulfur. Could biotin play a role here? Very little is known about cytochrome oxidase. Some of the vitamins might find a place in this compound or in some of the numerous dehydrogenases. However, it is more likely that they may find a role in the enzymes specifically concerned with fat and protein metabolism, or in the processes involving the resynthesis of pyruvic acid to glycogen. I may add that we are actively engaged in studying tissues from pyridoxine, pantothenic acid, and biotin deficient animals, but to date we have found no interesting leads.

Vitamin C was associated with enzymes long before it was isolated, but as far as I know no one has proved conclusively that it may be a constituent part of an enzyme, although it has been suggested as a prosthetic group in liver esterase by Kraut et al. (49).

Both ascorbic acid and dehydroascorbic acid exert stimulatory or inhibitory effects on many of the proteolytic and oxidizing enzymes. Meiklejohn and Steward (50) have recently shown that the ascorbic acid oxidase is a true copper protein compound and is much more active than an equivalent amount of ionic copper. Crook (51) has produced evidence that a specific enzyme is involved in the reduction of dehydroascorbic acid by glutathione.

When we turn to the fat soluble vitamins we find only indirect relationships. Both Green (52) and Sure et al. (53) have reported decreases in the blood and liver esterases during vitamin A deficiency. A more specific relation of vitamin A and enzymes comes from the work of Wald that visual purple is a conjugated protein with vitamin A as the prosthetic group. Whether vitamin A functions as a prosthetic group with proteins in other tissues is, of course, unknown. A high plasma phosphatase in vitamin D deficiency has been recognized for 10 years, but this too is undoubtedly a secondary effect due to loss of phosphatases from the rachitic bones.

Finally we may mention very briefly the relation of enzymes and para-aminobenzoic acid, which Ansbacher (54) has classified as a vitamin. Although we have been unable to demonstrate any effect of para-aminobenzoic acid in our experimental animals, it does function in the growth of certain microorganisms and it has been isolated from vitamin-rich material such as yeast and liver. Wisansky, Martin and Ansbacher (55) have shown that para-amino-benzoic acid retards the oxidation of tyrosine and dopa by tyrosinase. Lipman (56) has reported that para-aminobenzoic acid is oxidized by H_2O_2 and peroxidase and that this reaction is inhibited by sulfanilamide. It is also oxidized by phenol oxidase in the presence of catechol. This reaction is not inhibited by sulfanilamide.

In conclusion, I believe it is evident from this short review that studies on the relation of vitamins to enzymes have been the most profitable approach to an understanding of the biological action of vitamins. Since our knowledge of enzyme systems in normal tissues is still far from complete, further studies in this field will aid us in explaining the metabolic disturbance during avitaminosis. We should keep in mind, however, that all the vitamins may not function in this manner and that the other approaches to the problem should be continued.

REFERENCES

(1) Eijkman, C. Arch. Path. Anat. **149:** 187, 1897. (2) Hart, E. B., E. V. McCollum, H. Steenbock and G. C. Humphrey. Wisconsin Agricultural Exper. Sta. Bull. 17, 1911. (3) Hart, E. B., H. Steenbock, G. C. Humphrey and R. S. Hulce. J. Biol. Chem. **62:** 315, 1924. (4) Goldberger, J., C. H. Waring and D. G. Willets. Pub. Health Repts. **30:** 3117, 1915. (5) Boas, M. A. Biochem. J. **21:** 712, 1927. (6) Parsons, H. T. and E. Kelly. J. Biol. Chem. **100:** 645, 1933. (7) György, P., C. S. Rose, R. E. Eakin, E. E. Snell and R. J. Williams. Science **93:** 477, 1941. (8) Daft, F. S., W. H. Sebrell, S. H. Babcock, Jr. and T. H. Jukes. Pub. Health Repts. **55:** 1333,

1940. (9) SEIDELL, A. J. Ind. Eng. Chem. **13:** 72, 1921. (10) LOHMANN, K. AND P. SCHUSTER. Biochem. Ztschr. **294:** 188, 1937. (11) NEUBERG, C. AND A. HILDESCHEIMER. Biochem. Ztschr. **31:** 170, 1911. (12) AUHAGEN, E. Hoppe-Seyler's Ztschr. **204:** 149, 1932. (13) GREEN, D. E., D. HERBERT AND V. SUBRAHMANYAN. J. Biol. Chem. **138:** 327, 1941. (14) PETERS, R. A. AND R. H. THOMPSON. J. Biol. Chem. **28:** 916, 1934. (15) LIPTON, M. A. AND C. A. ELVEHJEM. Cold Spring Harbor Symposium Quant. Biol. **7:** 184, 1939. (16) OCHOA, S. AND R. A. PETERS. Biochem. J. **32:** 1510, 1938. (17) GOODHART, R. AND H. M. SINCLAIR. J. Biol. Chem. **132:** 11, 1940. (18) SIMOLA, P. E. Biochem. Ztschr. **254:** 229, 1932. (19) GREEN, D. E., W. W. WESTERFELD, B. VENNESLAND AND W. E. KNOX. J. Biol. Chem. **141:** 683, 1941. (20) BARRON, E. S. G., C. M. LYMAN, M. A. LIPTON AND J. M. GOLDINGER. J. Biol. Chem. **141:** 957, 1941. (21) MANN, P. J. G., J. H. QUASTEL. Nature **145:** 856, 1940. (22) BERNHEIM, F. J. Lab. Clin. Med. **26:** 20, 1940. (23) WARBURG, O. AND W. CHRISTIAN. Biochem. Ztschr. **254:** 438, 1932. (24) HAAS, E. Biochem. Ztschr. **298:** 378, 1938. (25) DEWAN, J. G. AND D. E. GREEN. Biochem. J. **32:** 626, 1938. (26) EULER, H. V. AND H. HELLSTRÖM. Ztschr. physiol. Chem. **252:** 31, 1938. (27) STRAUB, W. Biochem. J. **33:** 787, 1939. (28) HAAS, E., B. L. HORECKER AND T. R. HOGNESS. J. Biol. Chem. **136:** 747, 1940. (29). ALTSCHUL, A. M., H. PERSKY AND T. R. HOGNESS. Science **94:** 349, 1941. (30) WARBURG, O. AND W. CHRISTIAN. Biochem. Ztschr. **296:** 294, 1938. (31) AXELROD, A. E., H. A. SOBER AND C. A. ELVEHJEM. J. Biol. Chem. **134:** 749, 1940. (32) OCHOA, S. AND R. J. ROSSITER. Biochem. J. **33:** 2008, 1939. (33) KLEIN, J. R. AND H. I. KOHN. J. Biol. Chem. **136:** 177, 1940. (34) BALL, E. G. J. Biol. Chem. **128:** 51, 1939. (35) CORRAN, H. S., J. G. DEWAN, A. H. GORDON AND D. E. GREEN. Biochem. J. **33:** 1694, 1939. (36) AXELROD, A.E. AND C. A. ELVEHJEM. J. Biol. Chem. **140:** 725, 1941. (37) SURE, B. AND M. DICHEK. J. Nutrition **21:** 453, 1941. (38) LWOFF, A. AND M. LWOFF. Proc. Royal Soc., London **122:** 352, 1937. (39) WEST, R. AND A. F. COBURN. J. Exper. Med. **72:** 91, 1940. (40) WEST, R. Proc. Soc. Exper. Biol. Med. **46:** 369, 1941. (41) JANDORF, B. J., F. W. KLEMPERER AND A. B. HASTINGS. J. Biol. Chem. **138:** 311, 1941. (42) KATZENELBOGEN, E., A. E. AXELROD AND C. A. ELVEHJEM. J. Biol. Chem. **141:** 611, 1941. (43) HANDLER, P. AND W. J. DANN. J. Biol. Chem. **140:** 739, 1941. (44) MANN, P. J. G. AND J. H. QUASTEL. Biochem. J. **35:** 502, 1941. (45) KOHN, H. I., J. R. KLEIN AND W. J. DANN. Biochem. J. **33:** 1432, 1939. (46) AXELROD, A. E., R. J. MADDEN AND C. A. ELVEHJEM. J. Biol. Chem. **131:** 85, 1939. (47) AXELROD, A. E., T. D. SPIES AND C. A. ELVEHJEM. J. Biol. Chem. **138:** 85, 1939. (48) KOHN, H. I. AND J. R. KLEIN. J. Biol. Chem. **130:** 1, 1939. (49) KRAUT, H. AND W. PANTSCHENKO-JUREWITZ. Biochem. Ztschr. **275:** 114, 1935. (50) MEIKLEJOHN, G. T. AND C. G. STEWARD. Biochem. J. **35:** 755, 1941. (51) CROOK, E. M. Biochem. J. **35:** 226, 1941. (52) GREEN, H. N. Biochem. J. **28:** 16, 1934. (53) SURE, B., M. C. KIK, K. S. BUCHANAN, R. T. HARRELSON, JR. AND R. M. THEIS. Arkansas Agric. Exp. Sta. Bull. 373, 1939. (54) ANSBACHER, S. Science **93:** 164, 1941. (55) WISANSKY, W. A., G. J. MARTIN, AND S. ANSBACHER. J. Am. Chem. Soc. **63:** 1771, 1941. (56) LIPMAN, F. J. Biol. Chem. **139:** 977, 1941.

VITAMINS AS PHARMACOLOGICAL AGENTS

HANS MOLITOR

Merck Institute for Therapeutic Research, Rahway, N. J.

A discussion of vitamins as pharmacological agents appears at first to be a rather uninteresting undertaking, since in comparison with other potent remedies these substances fail to produce conspicuous pharmacodynamic effects. Indeed, the *range* between the therapeutic and the lethal dose is so wide, that one might feel justified to place vitamins in the same category as salt, sugar, or other basic food elements which are essential from the nutritional viewpoint, but practically inert in the pharmacological sense. However, such an opinion would be erroneous since vitamins are pharmacologically quite active, provided they are investigated in animals suffering from a specific vitamin deficiency.

The fact that a drug, in order to exhibit its pharmacodynamic effect, has to be tested under certain pathological conditions, is not unusual. Antipyretics, spasmolytics and analgesics may be cited as other examples. However, while the pathological condition necessary for demonstrating the effect of these drugs may be produced in a variety of ways, a vitamin affects only those conditions which result from a deficiency of that particular agent; in this respect, vitamins resemble in their therapeutic specificity the immune sera

or antitoxins. Failure to recognize this accounts not only for a large number of ill-founded therapeutic claims, but also for many of the disappointments of vitamin therapy and the skepticism originating therefrom.

It can readily be seen, that a discussion of vitamins as pharmacological agents must distinguish between their effect in the normal and in the vitamin deficient animal. In normal animals their action is rather unspecific and pharmacodynamic effects are produced only by doses several thousand times larger than those needed for a therapeutic effect (table 1).

The only vitamin which produces a distinct pharmacologic effect even in therapeutic doses, is nicotinic acid. Its administration is followed in humans by a transitory peripheral vasodilatation. However, this effect cannot be ascribed to its nature as a vitamin, since it fails to occur on the administration of nicotinamide, which is equally effective as a vitamin.

TABLE 1

Lethal doses of B vitamins as compared with their daily requirement

	SPECIES	DAILY REQUIREMENT	L.D. 50 ORAL	RATIO
Thiamin	mouse	3γ	100 mgm.	30,000
Riboflavin	mouse	3γ	>7 mgm.	>2,000
Nicotinic acid	dog	0.25 mgm.[1]	>2 grams[1]	>8,000
Pyridoxine	rat	10γ	1 gram[2]	100,000
Pantothenic acid	rat	100γ	>2 gram[2]	>20,000

[1] Per kilogram body weight.
[2] Per rat (200 grams body weight).

With therapeutic margins of from 1:1000 upward, the danger of *acute* toxicity is obviously of very minor concern to practical vitamin therapy. The question of *chronic* toxicity deserves more attention since vitamins, due to their nature as nutritional factors, are likely to be taken over a prolonged period of time and without supervision by a physician. Fortunately, all information presently available indicates that the danger of chronic hypervitaminosis is practically nonexistent. Daily feeding of up to several hundred times the maintenance doses of thiamin, riboflavin, pyridoxine, nicotinic acid and pantothenic acid over the entire life span of rats fails to produce toxic effects. However, since pathologic changes resulting from prolonged administration of large doses of vitamins may become manifest only in the second generation, toxicity experiments should be extended beyond the first generation. Thus, Perla (1) has shown that in rats prolonged administration of thiamin causes in the third generation loss of maternal instinct and interferes with lactation; Sure (2) has made similar observations and we obtained such findings in an experiment extended over 4 generations.

According to Perla (3), this toxicity is due to a relative lack of manganese in the diet and can be prevented by addition of traces of this element. Thus, ordinarily innocuous doses of vitamins may become harmful under certain conditions. Unfortunately, not much experimental data is as yet available to elaborate on this point. V. A. Drill (4) reported, that hyperthyroid female rats, which had lost weight and were still receiving a thyroid preparation, regained their weight when both thiamin and vitamin B complex were administered; and Sure and Smith (5) found that in such animals the weight loss could be retarded by administration of a potent vitamin B concentrate. In a later paper, Sure and Buchanan (6) state that pure thiamin in daily doses of 30 to 100 micrograms counteracts the toxic effects of 0.2 mgm. thyroxine daily. In our laboratory we have

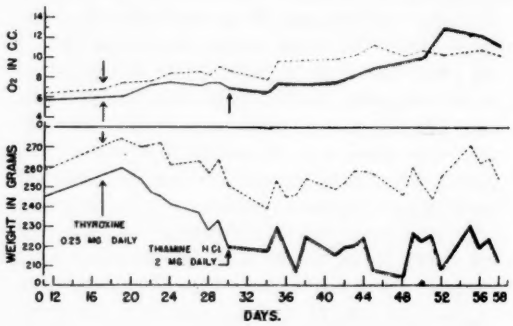


Fig. 1. Effect of thiamine on weight and O_2 consumption of hyperthyroid rats. ..... indicates the average weight. Curves represent the averages of a group of 4 rats. All animals received 0.25 mgm. thyroxine and those represented in the lower curve received 2 mgm. thiamin hydrochloride at the point indicated.

investigated the influence of hyperthyroidism on the toxicity of very large doses of thiamin. Rats were given daily doses of thyroxine and simultaneously, or after establishment of a hyperthyroid state, large amounts of thiamin were administered. In addition to daily determination of the oxygen consumption and body weight frequent records were taken of the heart and respiratory rate and the body temperature. Although more than 200 rats were used in these experiments, the normal and hyperthyreotic rats did not differ significantly in their sensitivity to thiamin (fig. 1).

A question of practical importance is whether an imbalance in a combination of vitamins may lead to toxic effects which are not observed if the vitamins are given in their naturally occurring mutual relationship. This problem has attained particular prominence since the enrichment of foods with selected members of the vitamin B

complex has become an established practice, although even those investigators who believe in the possibility of an increased vitamin toxicity due to imbalance have always emphasized, that this danger does not exist for the relatively minute quantities of vitamins incorporated into flour, bread or other foodstuffs. The only published experimental data indicating an adverse effect of vitamin imbalance are those of A. F. Morgan (7), who showed that dogs suffering from a combined nicotinic acid and pantothenic acid deficiency lived longer than those on nicotinic acid or pantothenic acid deficiency alone.

In our laboratory the effect of pantothenic acid or riboflavin was studied in adult dogs during a combined pantothenic acid-riboflavin deficiency (table 2). No increase in toxicity of either vitamin was found in these conditions. Whether the dogs were on the combined deficiency or only on riboflavin deficiency, the same length of time

TABLE 2

Progress of depletion symptoms in riboflavin and riboflavin-pantothenic acid deficient dogs

DOG NO.	DEFICIENCY	COLLAPSE AFTER	RIBOFLAVIN EXCRETION IN γ/24 HOUR				
			1st week	15th week	22nd week	28th week	29th week
		weeks					
108	Riboflavin	23	77	15	*6*		
115	Riboflavin	29	30	15	17	*8*	
103	Riboflavin and Pantothenic Acid	30	175	28	27	36	*11*
113	Riboflavin and Pantothenic Acid	22	37	12	*8*		

was required to produce collapse and the precollapse level of riboflavin in the urine was essentially the same in both groups.

It has been reported that during a multiple deficiency correction of only one component tends to aggravate the symptoms of the other deficiencies. Sydenstricker (8), Spies and others described the development of nicotinic acid and riboflavin deficiency symptoms in sub-acute cases of pellagra treated only with thiamin. György (9), Chick (10) and Harris (11) have shown that in rats maintained on diets deficient in both riboflavin and vitamin B_6, the dermatitis characteristic of the lack of the latter does not become manifest until riboflavin is supplied. Daft and Sebrell (12), and Lepkovsky, Jukes and Krause (13) have made similar observations on the appearance of pantothenic acid deficiency lesions in rats deficient both in pantothenic acid and pyridoxine. However, such observations must not necessarily be regarded as evidence for an adverse effect of a vitamin imbalance because they may merely indicate that some deficiencies require more time for development than others, time which is provided in these experiments through the cure of that deficiency which occurred first.

In a series of experiments undertaken in our laboratory, the effect of prolonged administration of large doses of single vitamins of the B group on the course of multiple deficiencies was investigated. There was no indication of an increased toxicity (14). Similarly, we have been unable to demonstrate a greater toxicity of thiamin in animals suffering from riboflavin deficiency (table 3).

As with other medicinal agents, the question of optimal dosage, most effective spacing and preferred way of administration deserves careful consideration.

It appears advisable to distinguish between a *maintenance* dose, sufficient to provide an adequate supply of the vitamin in a non-depleted organism and a *curative* dose, necessary to restore

TABLE 3

Acute toxicity of thiamine in normal and riboflavin deficient rats

MODE OF ADMINISTRATION	NORMAL RATS		RIBOFLAVIN DEFICIENT RATS	
	L.D. 0	L.D. 50	L.D. 0	L.D. 50
	mgm./kgm.	*mgm./kgm.*	*mgm./kgm.*	*mgm./kgm.*
S.C.	500	1400	>750	1500
P.O.	6000	9500	6000	9000

to normal a vitamin depleted animal. Obviously, the curative dose is usually much larger than the maintenance dose, making it advisable to administer the vitamin in its pure form and, quite frequently, by parenteral injection.

Contrasted to this strictly medicinal use is the equally essential use of vitamins for the maintenance of dietary balance. The doses required for this purpose are much smaller than those needed for therapy and can be supplied through a carefully selected diet or specially prepared and enriched foodstuffs.

Since there is practically no danger of overdosing with any of the vitamins, the principal points of interest are those related to absorption, storage and excretion as they have a direct bearing on the mode of administration. The various groups of vitamins differ greatly in these properties. Generally speaking, the fat soluble vitamins A and D are stored to a greater extent than the water soluble vitamins B and C. This influences not only the rapidity with which deficiencies may develop, but also the efficiency of different modes of administration.

As with other medicinal agents, the availability of a vitamin to the organism is determined by the extent to which it may be stored, absorbed, destroyed and excreted. Since all of these factors are closely interrelated, conclusions which are based on only one of them must be drawn with great reservation. Thus, the difference between the dose administered and excreted is not necessarily an indication of the basic requirement. For example, of a dose of 100 micrograms of pantothenic acid given to a rat, 7 micrograms were excreted. However, when 250 micrograms were given only 67 were excreted and with 3000 micrograms only 600 micrograms could be found in the urine. On the basis of these findings, the requirement would appear to be 93, 183 and 2400 micrograms respectively, while actually it is about 100 micrograms. The difference in the foregoing findings must be attributed either to storage, non-absorption or destruction; as a rule each of these factors plays a rôle.

TABLE 4

Oral and intravenous toxicity of B vitamins in rats

	INTRAVENOUS	PERORAL
	gm./kgm.	*gm./kgm.*
Thiamin	0.170	9.5
Riboflavin	0.560	more than 10
Nicotinamide	2.2	3.5
Pyridoxine	0.657*	5.5
Pantothenic acid	0.830	more than 10

* Weigand, Eckler and Chen, Proc. Soc. Exp. Biol. & Med., **44**, 147, 1940.

Vitamins being essential food elements, they can obviously be effectively administered by mouth. However, under certain pathological conditions, such as severe gastro-intestinal disturbances, their absorption may be greatly delayed or even completely absent and in these cases parenteral administration is indicated.

Administration of vitamins by other than the peroral route influences the relative toxicity as well as the efficacy. As with most other drugs, the acute intravenous toxicity is generally considerably greater than the subcutaneous, and in turn, the peroral (table 4). Furthermore, the intravenous toxicity depends much upon the rate of injection (table 5). Whether the *intravenous* or *subcutaneous administration* of a vitamin is therapeutically more effective than its peroral administration depends upon several factors of which absorption from the gastrointestinal tract is only one. Intravenous injection produces not only (though temporarily) a much higher concentration of the vitamin in the blood, but probably also in the tissues. Thus, intravenous injection of 4 mgm. per kgm. of riboflavin to dogs in the form of its sodium salt raises the blood level 15 to 20 times above that obtained by feeding an excess dose by mouth; and the riboflavin content in the liver of rats increased from 35 micrograms per gram to 160 micrograms per gram after a dose of 500 mgm. per kgm.

The fact that parenteral administration can raise the concentration of a vitamin in the blood and tissues far above that obtainable by feeding of even the largest doses may explain certain therapeutic successes obtained by clinicians through parenteral administration of relatively enormous doses of vitamins. Furthermore, with

TABLE 5

Influence of rate of injection on intravenous thiamine toxicity

DOSE	NO. OF ANIMALS	VOLUME INJECTED	CONCENTRATION OF SOLUTION	RATE OF INJECTION	NO. DEAD
mgm./kgm.		*c.c.*	*per cent*	*cc./min.*	
90	10	0.25	0.72	0.1	1
90	10	0.25	0.72	0.2	5
90	10	0.25	0.72	0.4	7

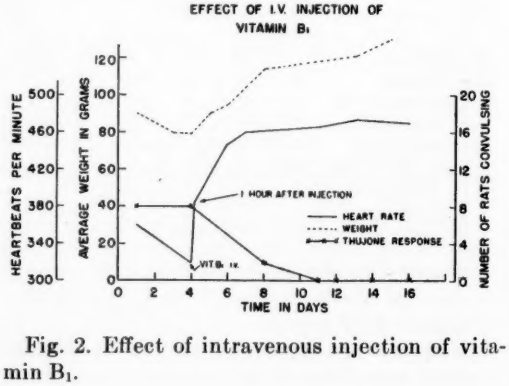


Fig. 2. Effect of intravenous injection of vitamin B_1.

this mode of administration the pharmacodynamic effect is likely to take place with great rapidity. Thus, the effect of thiamin on bradycardia due to deficiency of this vitamin can be observed within less than 1 hour (fig. 2). Still more dramatic is the life-saving effect of intravenously injected vitamins to animals in collapse from pantothenic acid or riboflavin deficiency or to beriberi patients suffering from acute cardiac decompensation. However, even when given by mouth, the therapeutic effect of most vitamins takes place very rapidly, provided the absorption from the gastro-intestinal tract is still normal.

While certain therapeutic effects may be more readily achieved by parenteral, particularly

intravenous administration, this procedure should probably be reserved for specific indications, since it introduces some of the hazards common to all injections made directly into the blood stream. One of these is anaphylactic shock. Rare cases of such incidents have been reported following the intravenous injection of thiamin. In our laboratory we have investigated the sensitizing effect of thiamin in dogs and guinea pigs. Ten dogs were divided into two groups. One group received on 10 consecutive days 50 mgm. per kgm. of thiamin hydrochloride intravenously, an amount sufficient to produce severe toxic reactions such as excessive salivation, nausea, vomiting and collapse. After 10 daily injections, an interval of 10 to 60 days was allowed before the final experiment was performed. The second group of dogs received no pretreatment. There was no significant difference between the two groups. However, upon autopsy it appeared that in some of the dogs which had received previously 10 doses of thiamin, the right heart was dilated and

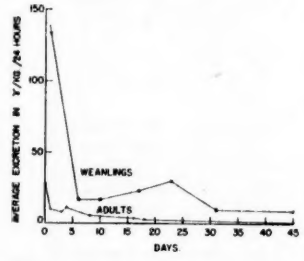


Fig. 3. Urinary excretion of pantothenic acid in weanling and adult dogs during depletion.

the abdominal organs showed greater congestion, while this was not observed in any of the controls. The outcome of the guinea pig experiments was not conclusive.

Normal as well as depleted animals excrete a certain amount of the administered vitamin through the urine. Both the absolute amount and the percentage of excretion (with or without administration of a test dose) have been used as a measure of the state of relative saturation and as indicator of the degree of deficiency. It is in the urine, where the first sign of vitamin depletion can usually be found (fig. 3). However, it must be remembered, that without the use of a test dose the urinary level may merely be a measure of the immediately preceding vitamin intake. In order to be significant, such "saturation tests" should therefore be conducted in fasting animals and after administration of a test dose.

In contrast to the wide fluctuations in the vitamin content of the urine, the concentration in the blood remains usually remarkably constant and such determinations offer therefore little help in the diagnosis of early vitamin deficiencies.

While the difference between the amount of a vitamin administered and excreted is not necessarily an indicator of the *maintenance* dose, it can nevertheless serve as a guide for the determination of the appropriate *therapeutic* dose. This is shown in the following experiment, in which the same dose of pantothenic acid was daily given to pantothenic acid depleted dogs and its urinary excretion determined. It may be seen that at least 4 to 16 times the maintenance dose (previously determined in actual feeding experiments) had to be given over a period of 1 to 2 weeks until saturation was accomplished. This again emphasizes the justification for apparently excessive vitamin doses in cases of advanced deficiencies.

Other factors which may considerably affect the dosage of vitamins are diet and age. For example, thiamin is necessary for the utilization of carbohydrates and consequently larger doses are needed when the carbohydrate intake is high.

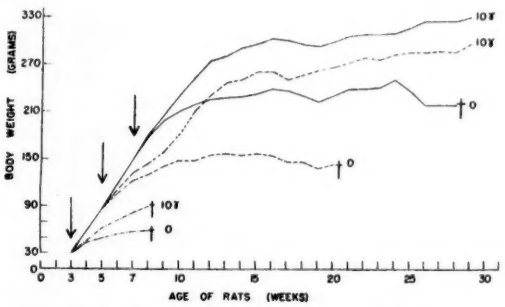


Fig. 4. Influence of age on pantothenic acid requirement. ↓ indicates when groups were changed from full diet to depletion diet. † indicates death of 50 per cent of the animals in group.

Similarly, it has been reported that the rat requirement for riboflavin or pyridoxine increases when a high fat diet is given.

The influence of age upon vitamin requirements depends upon the specific vitamin studied. Generally, a growing organism requires relatively larger amounts than an adult. This is particularly true for pantothenic acid where with increasing age a striking decrease takes place not only in the relative, but also in the absolute requirement (15) (fig. 4).

In the preceding paragraphs the general toxicity of vitamins and their absorption, storage and excretion have been discussed, but nothing has as yet been said about their pharmacodynamic properties. The reason for this is that in healthy animals vitamins in doses below the toxic range fail to exhibit such effects. Nevertheless, in a vitamin depleted animal they are capable of restoring to normal the pathological changes caused by lack of a specific vitamin and under

such conditions they compare in potency favorably with the most powerful medicinal agents, having a marked effect in doses within the milligram range.

The high specificity of vitamins can easily be demonstrated by comparing their effect upon pathological changes which, although closely resembling each other, are due in one instance to a specific vitamin deficiency and in the other to a non-specific cause. This is illustrated in the following experiment (fig. 5): Of two groups of rats one was placed on a thiamin deficient diet, while the other receiving a complete diet was restricted in its food intake to that of the depletion group. In both groups frequent records were taken of the weight, the heart rate and the sensitivity to a convulsing dose of thujone. It may be seen that in both groups convulsions could be produced more readily as the experiment progressed. However, only in the thiamin deficient group did the heart rate show the typical retarda-

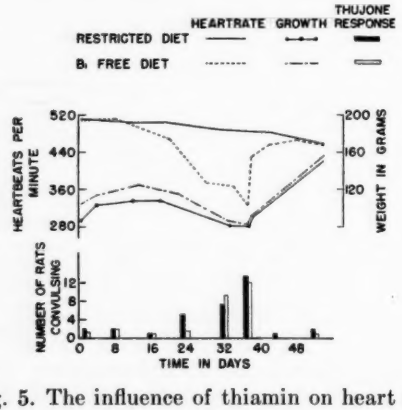


Fig. 5. The influence of thiamin on heart rate, growth and response to thujone.

tion, which responded immediately to thiamin administration. The increased sensitivity to the convulsant drug was, on the other hand, a result of the general state of inanition and responded therefore in both groups with equal promptness to an increased intake of food (16).

The observation of A. Keys (17) that administration of vitamins is without influence on the performance of athletes already living on an adequate diet, can be cited as further evidence that vitamins exhibit their effect only in specific deficiencies. However, if the subjects in Keys' experiment would have lived on a suboptimal vitamin intake, a beneficial effect of vitamins on the endurance might have been found. In our laboratory such an effect could be demonstrated in vitamin B_1 deficient rats. In experiments still in progress the endurance of rats in various stages of vitamin deficiencies is being measured by their ability to carry a given load while swimming. During thiamin deficiency, the work performance decreases to a very low level; administration of the vitamin improves it greatly, even if the food intake remains restricted (table 6).

A decrease in endurance due to a suboptimal intake of vitamins is but one of the many apparently non-specific conditions in which administration of vitamins may prove to be an effective therapeutic measure. While the clinical symptomatology of fully developed vitamin deficiencies, such as rickets, scurvy, beriberi and pellagra, is well known, there are undoubtedly a far greater number of subclinical deficiencies and of other

TABLE 6

Effect of thiamin-depletion on endurance

RAT NO.	WEIGHT	DATE	SWIMMING TIME	MEDICATION	REMARKS
	grams		*minutes*		
112	36	1/28	0	10γ Thiamin 4 gm. food	Fed and dosed after swimming
	35	1/29	12	4γ Thiamin 5 gm. food	
	36	1/30	14	4γ Thiamin 5 gm. food	
114	49	1/28	0	10γ Thiamin 4 gm. food	Fed and dosed after swimming
	49	1/29	16	4γ Thiamin 5 gm. food	
	50	1/30	60+	4γ Thiamin 5 gm. food	
119	52	1/28	0	10γ Thiamin 4 gm. food	Fed and dosed after swimming
	53	1/29	60+	4γ Thiamin 4 gm. food	
	56	1/30	60+	4γ Thiamin 4 gm. food	
SFE-1	53	3/21	6	10γ Thiamin food ad lib.	Control
	60	3/24	4	4γ Thiamin food ad lib.	
	62	3/25	4	4γ Thiamin food ad lib.	
SFE-2	62	3/21	11	10γ Thiamin food ad lib.	Control
	73	3/24	10	4γ Thiamin food ad lib.	
	76	3/25	16	4γ Thiamin food ad lib.	

pathological conditions, in which vitamin deficiency is a complicating factor. The latter respond to vitamin therapy to the extent to which the deficiency is eliminated. Thus, it has frequently been stated that thiamin administration in diabetes mellitus permits a reduction in the necessary dosage of insulin. However, in strictly controlled experiments it can be shown that thiamin itself does not influence the course of the disease; its beneficial effect is probably due to the correction of a mild deficiency which is often produced by imposition of a rigid diet. Similarly, administration of vitamins to patients with gastric ulcers or on other restricted diets may be indicated and may improve the general condition by correcting a nutritional deficiency.

TABLE 7

Pantothenic acid and riboflavin contents of livers of mice infected with influenza

INFECTED MOUSE	MOUSE WEIGHT	PANTOTHENIC ACID	RIBOFLAVIN	INANITION CONTROL	MOUSE WEIGHT	PANTOTHENIC ACID	RIBOFLAVIN
	grams	*γ per gram*	*γ per gram*		*grams*	*γ per gram*	*γ per gram*
No. 1	17.8	60	31	No. 21	15.5	76	28
2	17.3	71	33	22	17.5	92	29
3	14.4	59	40	23	13.3	79	36
4	15.5	69	34	24	13.5	86	29
5	19.2	60	28	25	18.7	96	32
6	9.3	61	30	26	9.1	80	32
7	14.6	62	29	27	10.3	79	29
8	14.6	56	30	28	10.5	63	24
9	19.5	69	32	29	18.9	83	25
10	15.4	58	25	30	12.1	87	29
11	12.5	59	26	31	10.8	65	27
12	11.9	51	30	32	10.3	79	36
13	16.0	46	21	33	12.6	66	28
14	15.2	49	28	34	12.9	104	30
15	15.5	49	33	35	13.0	94	32
16	17.3	58	33	36	12.2	88	31
17	17.1	58	27	37	15.9	77	25
19	13.8	54	31	39	10.5	96	34
20	11.9	53	32	40	9.5	69	26
Averages....		58	31			82	30

Under certain conditions, as in lactation, pregnancy, tropical environments (increased thiamin excretion through sweating) and infectious diseases, the vitamin requirements are considerably greater than normal. Thus, it has been reported by Mills (18) that the thiamin requirements of rats kept in a tropical climate are about twice those of the control animals. This is due primarily to a decreased food consumption in the hot room and can be corrected by doubling the thiamin concentrations in the diet. Mice on a sub-optimal riboflavin intake are less resistant to pneumococcus I infection (19); and in our own laboratory it has been found that during an infection with influenza virus the pantothenic acid content of the liver is significantly reduced, while the riboflavin content remains constant (table 7).

In summary, it may be stated that vitamins are pharmacological agents of extremely low toxicity which exhibit a pharmacodynamic effect only in an animal depleted of the specific vitamin. However, since mild stages of vitamin deficiencies are quite frequent and may accompany pathological conditions resulting from other causes, vitamins have a wide field of therapeutic application.

Acknowledgment. Much of the material presented in this report is unpublished data collected by Drs. A. O. Seeler, R. H. Silber, Mr. Michael Kniazuk, and Mrs. A. O. Edison of the Merck Institute staff. I take this opportunity to gratefully acknowledge their contributions.

REFERENCES

(1) Perla, D. Proc. Soc. Exper. Biol. and Med. **37:** 169, 1937. (2) Sure, B. J. Nutrition **19:** 57, 1940. (3) Perla, D. Proc. Soc. Exper. Biol. and Med. **41:** 522, 1939. (4) Drill, V. A. Proc. Soc. Exper. Biol. and Med. **39:** 313, 1938. (5) Sure, B. and M. Smith. J. Nutrition **7:** 547, 1934. (6) Sure, B. and K. S. Buchanan J. Nutrition **13:** 513, 1937. (7) Morgan, A. F. Science **93:** 261, 1941. (8) Sydenstricker, V. P. Ann. Internal Med. **15:** 45, 1941. (9) György, P. Biochem. J. **29:** 741, 1935. (10) Chick, H., A. M. Copping and C. E. Edgar. Biochem. J. **29:** 722, 1935. (11) Harris, L. J. Biochem. J. **29:** 776, 1935. (12) Daft, F. S. and W. H. Sebrell Public Health Reports **54:** 2247, 1939. (13) Lepkovsky, S., T. H. Jukes and M. E. Krause. J. Biol. Chem. **115:** 557, 1936. (14) Unna, K. and J. D. Clark. J. Am. Med. Sciences (in press). (15) Unna, K. and G. V. Richards. J. Nutrition (in press). (16) Molitor, H. and W. L. Sampson. J. Physiol. **119:** 377, 1937. (17) Keys, A. and A. F. Henschel. J. Nutrition **23:** 259, 1942. (18) Mills, C. Am. J. Physiol. **133:** 525, 1941. (19) Woolley, J. G. and W. H. Sebrell. Public Health Report, January, 1941.

VITAMINS IN THE PRACTICE OF MEDICINE

NORMAN JOLLIFFE AND ROBERT GOODHART

New York University College of Medicine

There can be no doubt but that vitamins have a definite place in the practice of medicine. A question does exist concerning the relative importance of deficiencies of the vitamins, and other nutritional factors, in the causation of ill health, not only in individual patients, but in large groups of our population.

Unfortunately, and in spite of the fact that much has been written about the pros and cons of the problem, we are not in a position to make any categorical statements concerning the prevalence of vitamin deficiency states, other than that there is overwhelming evidence to indicate that they are much more commonly encountered, in the practice of medicine, than generally recognized. A more satisfactory understanding of the prevalence and importance of nutritional failure will ensue when the practicing physician becomes acquainted with and accustomed to looking for the various manifestations of this condition.

We consider nutritional failure present whenever adequate amounts of one or more nutritional factors fail to reach the internal environment of the body. This may arise through dietary inadequacy, in the production of which many factors may be involved. Inadequate diets result from poverty and poor food habits. The latter may be negative or positive. By negative poor food habits is meant the failure to ingest adequate amounts of nutritious foods. The ingestion of foods of little nutritional value, such as candy, soft drinks, and alcohol, constitutes positive poor food habits.

Dietary inadequacy may be the result of factors, other than poverty, over which the individual has little or no control. Thus the centralization of the milling industry with the concomitant improvements in milling processes, has resulted in a deterioration in the nutritional value of white flour; a fact only recently appreciated. Now, many such flours and breads are being enriched according to the specifications of the Food and Nutrition Board of the National Research Council.

Dietary inadequacy is inherent in some of the therapeutic diets prescribed by many physicians. An apparently adequate diet may become grossly inadequate as the result of increased metabolic requirements caused by increased working hours, increased severity of work or exercise, insufficient rest or sleep, disease states associated with fever, etc.

Nutritional failure is present whenever the individual fails to absorb from the intestinal tract an ingested essential food substance. Thus in obstruction of the biliary tract or pancreatic ducts, where there is difficulty in the splitting and absorption of fats, inadequate absorption of the fat soluble vitamins; A and its provitamin, carotene; vitamin K; and activated 7-dehydro cholesterol and activated ergosterol, may ensue. There is a failure of absorption of many essential food factors associated with such diseases as sprue, coeliac disease, and all conditions accompanied by diarrhea. Failure of absorption may result from such simple causes as the continued daily ingestion of mineral oil.

Failure of the body to utilize and store food substances following ingestion and absorption may be responsible for nutritional failure in some instances. Cirrhosis of the liver is accompanied by impairment in the utilization and storage of carotene and vitamin A in the body.

In other instances increased excretion may be a factor. The loss of large amounts of ascorbic acid and thiamin may be associated with excessive perspiration. Large amounts of the water soluble vitamins may be excreted in the urine in diabetes insipidus and uncontrolled diabetes mellitus.

The nutritional inadequacy resulting from any of the above causes sooner or later may be followed by malnutrition, the successive stages of which are tissue depletion, biochemical lesions, functional changes, and finally anatomic lesions (1).

In the management of nutritional failure, prophylaxis is extremely important. Wherever there is good reason to suppose that nutritional inadequacy will develop or it is found to exist, remedial procedures are indicated. It must be remembered that there are only seven vitamins the lack of which has been associated with definite clinical syndromes in man. There are many other accessory food factors about which the investigator can tell us little except that they are essential for life. Even in the case of the seven vitamins about which we know the most, we are largely unable to diagnose the biochemical lesions and functional abnormalities prior to the development of the more advanced anatomic changes.

In table 1 (1) are listed gross evidences of malnutrition within the power of every practising physician to detect and diagnose without the aid of laboratory facilities. Every sign in the table represents an anatomical change and therefore indicates a relatively advanced degree of malnutrition. All, however, when detected in their earliest clinical stages, are completely reversible with adequate therapy.

A few laboratory procedures of proven value for the detection of malnutrition are given in table 2 (2). None of the methods listed here require equipment or chemicals not available in any well equipped hospital or clinic. Methods, such as the measurement of dark adaptation, which depend upon subjective responses and methods for which satisfactory techniques or criteria for their interpretation seem not to have been established, are not included. As an aid in the diagnosis of riboflavin deficiency examination of the cornea with the biomicroscope has proven of definite value. There seems to be little doubt but that the extensive vascularization of Rosacea Keratitis is associated with ariboflavinosis and responds to adequate therapy with riboflavin. Evidence is accumulating however that not all increased vascularity of the limbus and cornea isnecessarily due to ariboflavinosis.

TABLE 1

Gross evidences of malnutrition

SYSTEM	FINDING	SUGGESTED DEFICIENCY OR SYNDROME
Eyes	Xerosis conjunctivae and cornea	Vitamin A
	Central ophthalmoplegia	Thiamine
Mucous membranes	Scarlet red stomatitis and glossitis with or without secondary Vincent's infection	Nicotinic acid
	Magenta glossitis	Riboflavin
	Atrophic glossitis	Nicotinic acid B-Complex Addisonian anemia Plummer-Vincent Synd.
	Scorbutic gums	Ascorbic acid
	Cheilosis	Riboflavin
	Non-specific urethritis, balanitis, vaginitis	Nicotinic acid
Skin	Pellagrous dermatitis	Nicotinic acid
	Seborrheic lesions in nasolabial folds on face, behind ears and in skin folds	Riboflavin
	Hyperkeratosis and hyper-folliculosis	Vitamin A
	Hemorrhagic manifestations	Vitamin K Ascorbic acid
	Fissures in angles of mouth	Riboflavin
Neurological	Characteristic bilateral symmetrical polyneuropathy	Thiamine
	Combined system syndromes	Thiamine B-Complex Addisonian anemia
	Wernicke's Syndrome	Thiamine B-Complex
	Nicotinic acid deficiency encephalopathy	Nicotinic acid
	Progressive stupor and hebetude	Nicotinic acid
	Certain organic reaction psychoses	Nicotinic acid, Thiamine B-Complex
Skeletal	Rachitic deformities and osteomalacia	Vitamin D Calcium Phosphorus
General	Underweight, underheight, edema, pallor	Calories Proteins Iron B-Complex

The prophylactic treatment of malnutrition consists not merely of the provision of amounts of foodstuffs, adequate in quantity and quality, but also insurance of their ingestion and their utilization by the body. This necessitates individualization in dietary prescription in many instances. Occasions frequently arise in the practise of medicine where the physician must take into account the dietary foibles of his patients. Conditions associated with increased metabolic requirements, such as fever, pregnancy, lactation, and hyperthyroidism, require special attention and increased allowances of essential food factors. Disease states, the management of which includes dietary restrictions, may require the administration of food concentrates or pure vitamins, minerals, carbohydrates, etc., if the nutritional health of the patient is to be maintained.

TABLE 2

Special examinations for detection of malnutrition

EXAMINATION	CONDITION IT MAY DETECT
Roentgenogram of hand and wrist, elbow and hip	Rickets and scurvy in children; osteomalacia and scurvy in adults
Roentgenogram of heart	Advanced beriberi
Electrocardiogram	Changes suggestive of thiamine deficiency
Biomicroscopic eye examination with slit lamp	Capillary invasion of cornea (riboflavin deficiency)
	Changes in conjunctivae (vitamin A deficiency)
Red blood cell count Hemoglobin Stained blood smear Red blood cell volume	Iron-deficiency anemia; Addisonian anemia; macrocytic anemia
Plasma ascorbic acid	Vitamin C undersaturation
Serum calcium Serum phosphatase Serum phosphorus	Vitamin D deficiency
Blood pyruvic acid	Thiamine deficiency
Serum protein or albumin	Protein deficiency
Blood prothrombin	Vitamin K deficiency

The logical treatment of malnutrition whether prophylactic or curative, begins with the provision of a diet which is as adequate in all respects as is possible of achievement. The recommended daily allowances of the Food and Nutrition Board for some of the essential food factors are shown

in table 3 (3). These should be regarded as the minimum requirements necessary to maintain good health. A diet which provides less than the listed amounts of one or more essential foodstuffs is definitely in need of supplementation. Where possible, supplementation in prophylactic therapy should take the form of natural foodstuffs or natural food concentrates, such as liver extracts, yeast, wheat germ, fish liver oil, etc. This is advisable in order to insure adequate supplies every meal with butter or fortified oleomargerine, with the remaining calories to be supplied by a choice of vitamin-rich foods. These are minimum daily requirements for the maintenance of good health.

Curative treatment is built upon, not substituted for, prophylactic therapy. The diet is made as adequate in all respects as is feasible. Use is made of supplementation with natural food concentrates to the tolerance of the patient.

TABLE 3

Recommended daily allowances for specific nutrients[1]

(Committee on Foods and Nutrition, National Research Council)

	CALORIES	PROTEIN	CALCIUM	IRON	VITAMIN A[2]	THIAMIN[3]	ASCORBIC ACID[3]	RIBOFLAVIN	NICOTINIC ACID	VITAMIN D[4]
		grams	*grams*	*mgm.*	*I.U.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*I.U.*
Man (70 kgm.)										
Moderately active	3000	70	0.8	12	5000	1.8	75	2.7	18	
Very active	4500					2.3		3.3	23	
Sedentary	2500					1.5		2.2	15	
Woman (50 kgm.)										
Moderately active	2500	60	0.8	12	5000	1.5	70	2.2	15	
Very active	3000					1.8		2.7	18	
Sedentary	2100					1.2		1.8	12	
Pregnancy (latter ½)	2500	85	1.5	15	6000	1.8	100	2.5	18	400–800
Lactation	3000	100	2.0	15	8000	2.3	150	3.0	23	400–800
Children up to 12 years[6]										
Under 1 year[5]	100/kgm.	3–4/kgm.	1.0	6	1500	0.4	30	0.6	4	400–800
1–3 years	1200	40	1.0	7	2000	0.6	35	0.9	6	
4–6 years	1600	50	1.0	8	2500	0.8	50	1.2	8	
7–9 years	2000	60	1.0	10	3500	1.0	60	1.5	10	
10–12 years	2500	70	1.2	12	4500	1.2	75	1.8	12	
Children over 12 years[6]										
Girls, 13–15 years	2800	80	1.3	15	5000	1.4	80	2.0	14	
16–20 years	2400	75	1.0	15	5000	1.2	80	1.8	12	
Boys, 13–15 years	3200	85	1.4	15	5000	1.6	90	2.4	16	
16–20 years	3800	100	1.4	15	5000	2.0	100	3.0	20	

[1] These are tentative allowances toward which to aim in planning practical dietaries. These allowances can be met by a good diet of natural foods; this will also provide other minerals and vitamins, the requirements for which are less well known.

[2] Requirements may be less than these amounts if provided as vitamin A, greater if chiefly as the provitamin carotene.

[3] 1 mgm. thiamin equals 333 international units; 1 mgm. ascorbic acid equals 20 international units (1 international unit equals 1 U.S.P. unit).

[4] Vitamin D is undoubtedly necessary for adults and older children; when not available from sunshine it should be provided probably up to the minimal amounts recommended for infants.

[5] Needs of infants increase from month to month. The amounts given are for approximately 6–18 months. The amounts of protein and calcium needed are less if from breast milk.

[6] Allowances are based on the middle age for each group, and for moderate activity.

of those food factors which as yet are not available in crystalline form.

The recommended daily allowances of the Food and Nutrition Board of the National Research Council require the daily consumption of *at least* one pint of milk; two servings of potatoes; two servings of fruit, one of which should be a citrus variety or tomato; two vegetables, one of which should be leafy, green or yellow; one egg; one serving of meat, fish or poultry; a whole grain cereal dish; whole grain or "enriched" bread at

However, the great advance in the therapy of malnutrition which has occurred during our lifetime has come about through the provision to the practising physician of synthetic, crystalline vitamins. Through the use of these substances, many people are now walking about in perfect health who otherwise would almost certainly be dead. The availability of crystalline vitamins has made it possible to administer these substances in amounts impossible of achievement with either natural foodstuffs or food concentrates.

The smallest effective therapeutic dose of any of the vitamins which has been studied sufficiently to allow such a statement, is from 5 to 10 times the quantity necessary to prevent the development of a deficiency disease. Therefore, full use should be made of the crystalline vitamins in the treatment of any deficiency disease for which they are available. Their use should always be in addition to the dietary therapy mentioned above.

There are a few general statements concerning their use which are applicable to all the vitamins:

1. Their action is specific for those bodily disturbances which result from their lack.
2. The daily therapeutic dose should be at least five times the maintenance requirement.
3. They can be given in amounts many times the maintenance requirements without untoward effects.
4. It is better to give too much than too little.
5. They are best given in divided doses throughout the day.
6. In the great majority of situations, oral administration is the method of choice.
7. Where oral administration is not feasible, hypodermic or intramuscular injection is to be preferred, when suitable preparations are obtainable.
8. Intravenous administration is effective but wasteful, as large quantities of the vitamin may be spilled in the urine, even in very deficient subjects.
9. No advantage has been demonstrated for the intrathecal injection of the vitamins in any condition.

The following daily therapeutic doses, for the seven vitamins which are associated with definite disease entities, are recommended:

Vitamin A	50,000 to 100,000 I.U.
Vitamin D	2,000 I.U.
Vitamin K	1 to 2 mgm.
Thiamin	10 to 300 mgm.
Riboflavin	10 to 50 mgm.
Nicotinic acid	100 to 1000 mgm.
Ascorbic acid	500 to 1,000 mgm.

The above dosages will be found effective in the treatment of most cases. In some instances larger amounts may be found necessary. The oral administration of the fat soluble vitamins should be accompanied by the administration of bile salts when obstruction to the free flow of bile into the duodenum is present. Where liver disease exists, vitamin A is preferable to carotene, due to the possible interference in the conversion of carotene, to active vitamin A. Nicotinic acid, when given orally, should be given as the amide, to avoid unpleasant side effects.

Prolonged prothrombin times caused entirely by intrinsic liver pathology cannot be expected to respond to therapy with vitamin K.

We can hope for increased understanding of nutritional failure when more reliable methods for the detection of biochemical, functional, and subgross anatomic lesions are developed. However, the general application in the practice of medicine of the facts now available will be a distinct advancement in the diagnosis and management of malnutrition.

REFERENCES

(1) JOLLIFFE, N., J. S. MCLESTER AND H. C. SHERMAN. J. A. M. A. **118:** 944, 1942. (2) JOLLIFFE, N. Chapter on Nutrition and the Deficiency Diseases in Preventive Medicine in Modern Practice. Paul B. Hoeber and Sons, New York. In press. (3) Recommended Allowances for Various Dietary Essentials, Committee on Food and Nutrition, National Research Council, J. Am. Diet. A. **17:** 565, 1941.

VITAMINS AND PUBLIC HEALTH

W. H. SEBRELL

Chief, Division of Chemotherapy, National Institute of Health, United States Public Health Service

The emergency which confronts us today demands that every possible effort be directed toward the successful prosecution of the war. The immediate criterion of value for all aspects of public health work and other branches of science should be the same as in industry—what is contributed toward the war effort. Progress in laboratory and clinical research has clarified the concept of the rôle of essential food elements in health and disease, but the knowledge gained in research must be applied in the prevention as well as the therapy of deficiency diseases if it is to be of value in the present emergency. The importance of the nutrition of the armed forces is acknowledged, but there is as yet little appreciation in the United States of the necessity for a good nutritional status of the defense worker who must build the weapons for the armed forces. The

civilian population is faced with new food problems resulting from the increased demand, decreased imports, increased exports, and rising prices.

The problem. Pellagra was recognized as a serious public health disease in the Southern United States as long ago as 1909. The work of Goldberger and his associates demonstrated that this disease was the result of poor diet and could be prevented and satisfactorily treated by diets containing the "pellagra preventive" food factor. Until the advent of nicotinic acid therapy, it was possible to use the number of deaths from pellagra as an index of the incidence of clinical cases. This is no longer feasible because of the efficacy of therapy and improved diagnosis of early cases. Published reports of clinical observations show that deficiency diseases are widespread both in geographical and numerical distribution. These reports are of patients presenting severe deficiencies, and there can be little doubt that the number of cases of recognized deficiency disease is small when compared with the number of persons with mild symptoms that do not force them to seek medical aid, or the manifestations are of such a slight degree that they are not recognized by the physician. The number of people in the United States who subsist on inadequate diets is difficult to determine. The highly publicized phrase of "45 million undernourished" is based on the report of Stiebeling and Phipard (1) of the Department of Agriculture. Their findings showed that 40 to 64 per cent of the families surveyed obtained "poor" diets. The survey was conducted in 43 industrial centers in eight major geographical regions and included wage-earners and low-salaried clerical workers. Although 45 million is a large figure even in these days, the standards used as criteria for judging the adequacy of the diets were lower than the standards which were later established by the National Research Council as representing an adequate diet. After evaluating the evidence on the prevalence of malnutrition based on dietary surveys, official mortality rates, hospital admission rates, and reports in the medical literature, Jolliffe, McLester and Sherman (2) recently concluded that malnutrition and dietary inadequacies are of frequent occurrence in this country.

Well-defined human clinical syndromes result from deficiencies of seven vitamins: Vitamin A, thiamine, niacin (nicotinic acid), riboflavin, vitamin C, vitamin D, and vitamin K. There is evidence that pyridoxine, biotin, p-aminobenzoic acid, choline and alpha tocopherol are also essential for humans. Since the various vitamins have been available in synthetic form, rapid progress has been made in the recognition of syndromes due to deficiencies. In an experimental situation similar to that used by Goldberger and Wheeler (3) to produce pellagra in humans by a restricted diet in 1915, Sebrell and Butler (4, 5) were able in 1938 to induce riboflavin deficiency and establish it as a separate clinical entity which is now recognized as being of widespread occurrence in this country. The lesions which were produced experimentally were as follows: Macerated linear fissures in the angles of the mouth, cheilosis, magenta colored tongue, and a seborrheic dermatitis involving the nasolabial folds and other parts of the face. Spies and his associates (6) reported that eye symptoms such as lacrimation, photophobia, and conjunctival injection occurred in some cases. Kruse, Sydenstricker, Sebrell and Cleckley (7, 8) have described the ocular lesions associated with ariboflavinosis as being characterized by vascularization of the cornea with the production of an interstitial keratitis. It should be noted that there is no proof that all vascularization of the cornea is due to riboflavin deficiency. Vascularization of the cornea is a non-specific symptom which may be due to a variety of causes and it is questionable whether one is justified in making a diagnosis of riboflavin deficiency on the basis of slight vascularity of the cornea as the only symptom. This subject is one which needs further investigation. Under experimental circumstances Williams and associates (9, 10) induced thiamine deficiency in experimental subjects in 1939 and described the multiplicity of resulting symptoms and signs. Experimental human scurvy was reported by Crandon, Lund, and Dill (11) in 1940. These experiments and the therapeutic use of the synthetic vitamins have permitted a more accurate delineation of the symptoms resulting from the deficiency of single vitamins. The therapeutic value of the synthetic vitamins has been demonstrated by their efficacy as agents in the treatment of syndromes previously considered as refractive to therapy. Recognition of the manifestations resulting from the specific deficiencies has served to emphasize the important concept that patients usually have a multiple rather than a single deficiency. This is well illustrated by the knowledge that some of the symptoms formerly considered part of the clinical picture of pellagra are in reality due to riboflavin and thiamine deficiencies complicating the predominating nicotinic acid deficiency. Therapy with synthetic vitamins in large doses is at times imperative in clinical practice. From the public health standpoint the need for such therapy is indicative of a failure to solve the problem of malnutrition. This problem will not be met by the use of vitamin pills and potions obtained across the drug counter. The public is constantly subjected to a barrage of pressure advertising

through the press and radio promoting the sale of vitamin preparations. Absurd claims suggesting health, vigor, and beauty have been successful, because the public spends millions of dollars yearly for vitamins not obtained on a physician's prescription. This money could be spent more wisely in the grocery store and would not only result in greater benefit to the purchaser, but would save vital materials used in the manufacture of vitamins.

Nutrition in industry. The influx of defense workers in some areas has overtaxed the restaurant and housing facilities so that workers have difficulty obtaining any type of meals, let alone meals that supply essential food elements. Workers have no knowledge of nutrition as a rule and their eating habits are poor. Dr. Robert S. Goodhart (12) of the Committee on Nutrition in Industry reported that in a West Coast plant with 5,000 workers most of the men came to work without any breakfast and every day a nearby restaurant served over a thousand breakfasts of coffee and doughnuts.

Studies are being conducted at the present time under the direction of Dr. F. G. Boudreau, Chairman of the Committee on Nutrition in Industry of the National Research Council, to evaluate the nutritional status of the defense workers and its effect on production. England learned by experience in the last war that the efficiency of the worker was increased if canteens in the factories supplied good meals. The larger establishments continued these canteens during peace time. Doctor Drummond (13) of the Ministry of Food reports that in England today every establishment employing over 200 workers must have a canteen which serves a mid-day meal that is designed to supply two-thirds of the daily requirements of essential food elements. Workers on the night shift, who for some reason do not like a large mid-shift meal, are furnished whole wheat bread sandwiches, cheese, milk, carrots, etc. These meals are sold at minimum price and supply the worker with food which he obtains without drawing on his ration allowances.

The clinical symptoms of vitamin deficiencies are quite well known, but our knowledge of the effects of partially deficient diets is not comprehensive. We need to know more of the influence of the nutritional status upon fatigue and efficiency in industry and its effect on absenteeism. Can workers be protected from certain occupational hazards by the use of specific food substances? Judging by the number of inquiries received by the Public Health Service, certain distributors of vitamin preparations are trying to sell employers their products to distribute to the workers by making claims that are without any basis, although a scientific basis is implied in the promotional material. Until a rational study is made of the need and effect of vitamin supplements it is unwise to use them under any except controlled experimental conditions. The employer is lulled into a false sense of security by following what he thinks is scientific opinion when he furnishes vitamin preparations. The need for adequate calories, protein, minerals, etc. does not come to his attention and is therefore neglected. If, instead of supplying vitamin preparations, an effort was made to utilize intelligently present dining room facilities or to establish facilities to furnish good food, the worker would not only get all the vitamins needed, but would get all the essential food elements. The establishment of plant dining rooms in the defense industries in this country could result in benefits to both the workers and the employer as well as the country. To be successful, however, the meals served must be intelligently planned, and properly prepared. Little or no choice of menu should be offered so the workman does not have to attempt to pick out the necessary foods that will give him a good meal. Limitation of variety would, in addition, decrease the cost of preparation, and make eating in the dining room attractive from this standpoint. There will always be many workers that carry the lunches to work with them. If the women who prepare the lunches can learn to prepare a lunch that will give the worker not only calories but other food essentials it will help meet the problem. The use of enriched bread can play a decisive rôle in this respect.

Enriched bread. The proportion of highly refined food in American diets has gradually increased in the past 50 years. This increase has resulted in a decrease in the vitamin and mineral content of the diet both by loss in refining and because refined foods have replaced other food sources which supplied essential food elements. In many respects the diet today is inferior in quality to that eaten by past generations. Ninety per cent of the flour sold today is white flour, a highly refined food that is a poor source of all vitamins and of most minerals unless enriched. An efficacious method of improving the average diet is to restore to such a widely used food the most important vitamin and mineral elements that were present in the natural product before milling. Through the coöperation of the National Research Council, government agencies and the milling and baking industries, enriched white flour and bread is now available. This flour and bread has been fortified by the restoration of thiamine, niacin (nicotinic acid), and iron. Limited production capacities have delayed the inclusion of riboflavin in this group of added substances. The selection of the substances to be restored was made on the basis of principles

adopted by the Council on Foods and Nutrition of the American Medical Association. The most common deficiency diseases in this country are those resulting from lack of niacin, thiamine, riboflavin, and iron, and the restoration of these essential food elements to this widely used staple food will make a convenient source available to all individuals at low cost. To be successful the enriched flour must include the cheaper grades that are purchased by the people who have the greatest need for extra supplements. There have been attempts to encourage the use of whole wheat bread which would obviate the need for fortification of white bread. This has been tried in this country, and recently an unsuccessful attempt was made in England, where, despite the expenditure of a large sum of money in an educational campaign, the people still demanded white bread because of the texture, appearance, and taste. White flour has better keeping and baking qualities than whole grain or undermilled grain products and the enrichment of flour has no effect on its physical characteristics or baking qualities. By eating an average amount of bread—six slices—an individual will obtain approximately one-fourth of the daily requirements of the added vitamins and minerals. Such an amount would be of little value from a therapeutic standpoint but would be of great value in the prevention of deficiency disease which is the real public health problem.

Nutrition in the civilian population. The food problem of our civilian population probably will never reach the critical state seen in European countries that face blockade and pillage, unless we refuse to put to a practical use the knowledge of nutrition we possess. The nutritional tragedies of the last world war and of Spain and the Nazi dominated countries of Europe today need never occur here even though we may be called upon to supply enormous quantities of food to our armed forces and to our allies, and importation of food is decreasing because of the war. We have the agricultural capacity to supply ourselves and allies with essential foods and can do so if the production of the right foods is promoted. England faces a blockade which is a major threat and has necessitated comprehensive rationing and full utilization of the foods available. Manipulation of available stocks, and ingenuity as characterized by the use of rose hips as a source of vitamin C, illustrates their method of meeting the situation. We have the problem of supply, but it is an economic problem as well as one of transportation. Rising prices, and rising income in some groups, make for greater distress among the wage earners who have a low fixed income, and disruption in non-defense industries has forced new unemployment.

The first attack on the nutrition problem must be directed toward the necessity of eating foods which supply the essential food elements. The rationing of sugar will have some benefits—calories previously obtained from highly refined vitamin-free sugar can be replaced by calories from sources such as vegetables, cereals and legumes which will supply minerals and vitamins in addition to the calories. The National Research Council has formulated a standard of allowances for the various dietary essentials as shown in table 1. These values are not absolute, but represent the best evidence on the subject that is available at the present time. On the basis of these values a "nutritional yardstick" was evolved which translates into everyday language the amounts and types of foods which should be eaten in order to obtain an adequate diet. The problem now is to get this information to the housewives who feed the nation and pack the lunch pails. The Division of Nutrition of the Office of Defense Health and Welfare Services has begun such an educational program on a nation-wide basis. This is carried on through local committees acting under state organizations. Nutrition aides are being trained and their duties will be to carry the information to every home so that there will be an opportunity for everyone to learn what he should eat and there will be no excuse for ignorance of the basis of a nutritionally sound diet. Economic problems will have to be handled by the local nutrition committees in accordance with local conditions and resources.

The food industry is aware that a successful effort to increase the nutritional knowledge of the housewife will result in increased business for the producers and distributors of necessary foods. The weight of their advertising has shifted from appeal on the basis of appearance and taste to emphasis on the value of the product in terms of vitamins, minerals, protein, etc. Foods rather than brands are advertised, and there is a spirit of cooperation and eagerness to have technically correct advertising which is most encouraging.

Long before the word vitamin was coined, food was known as one of the three necessities for survival. We know now that food can do more than just assure us of survival; we know that the right kind of food will protect us not only from incapacitating disease, but will furnish the essential elements necessary to reach the highest plane of metabolic efficiency. A knowledge of the rôle of vitamins in metabolism is a fundamental prerequisite for the establishment of any nutritional program. It is impossible to produce "supermen" by the feeding of vitamin pills, but it is possible to maintain normal individuals, and to prevent the "hidden" as well as the "hollow hunger" by making use of the knowledge we possess. Defi-

TABLE 1

Recommended daily allowances for specific nutrients*

Committee on Foods and Nutrition, National Research Council

	CALORIES	PROTEIN	CALCIUM	IRON	VITAMIN A***	THIAMIN (B_1)	RIBOFLAVIN	NICOTINIC ACID	ASCORBIC ACID	VITAMIN D
		grams	*grams*	*mgm.*	*I.U.*	*mgm.***	*mgm.*	*mgm*	*mgm.***	*I.U.*
Man (70 kgm.)										
Moderately active	3000	70	0.8	12	5000	1.8	2.7	18	75	* * *
Very active	4500					2.3	3.3	23		
Sedentary	2500					1.5	2.2	15		
Woman (56 kgm.)										
Moderately active	2500	60	0.8	12	5000	1.5	2.2	15	70	* * *
Very active	3000					1.8	2.7	18		
Sedentary	2100					1.2	1.8	12		
Pregnancy (latter half)	2500	85	1.5	15	6000	1.8	2.5	18	100	400–800
Lactation	3000	100	2.0	15	8000	2.3	3.0	23	150	400–800
Children up to 12 years:										
Under 1 year *	100/kgm.	3–4/kgm.	1.0	6	1500	0.4	0.6	4	30	400–800
1–3 years * *	1200	40	1.0	7	2000	0.6	0.9	6	35	* * *
4–6 years	1600	50	1.0	8	2500	0.8	1.2	8	50	
7–9 years	2000	60	1.0	10	3500	1.0	1.5	10	60	
10–12 years	2500	70	1.0	12	4500	1.2	1.8	12	75	
Children over 12 years:										
Girls, 13–15 years	2800	80	1.3	15	5000	1.4	2.0	14	80	* * *
16–20 years	2400	75	1.0	15	5000	1.2	1.8	12	80	
Boys, 13–15 years	3200	85	1.4	15	5000	1.6	2.4	16	90	* * *
16–20 years	3800	100	1.4	15	6000	2.0	3.0	20	100	

* Tentative goal toward which to aim in planning practical dietaries; can be met by a good diet of natural foods. Such a diet will also provide other minerals and vitamins, the requirements for which are less well known.

** 1 mgm. thiamin equals 333 I. U.; 1 mg. ascorbic acid equals 20 I. U.

*** Requirements may be less if provided as vitamin A; greater if provided chiefly as the pro-vitamin carotene.

* Needs of infants increase from month to month. The amounts given are for approximately 6–8 months. The amounts of protein and calcium needed are less if derived from breast milk.

* * Allowances are based on needs for the middle year in each group, (as 2, 5, 8, etc.) and for moderate activity.

* * * Vitamin D is undoubtedly necessary for older children and adults. When not available from sunshine, it should be provided probably up to the minimum amounts recommended for infants.

ciency disease in this country is not as spectacular as infectious disease because a subtle, insidious "5th column" attack is not as noticeable as the frontal assault by a familiar enemy. The results however are just as devastating—death, insanity, invalidism, or even more important from the viewpoint of public health and national welfare—impaired ability to produce.

REFERENCES

(1) STIEBELING, H. K. AND E. F. PHIPARD. Circular 507, U. S. Dept. of Agriculture, 1939. (2) JOLLIFFE, N., J. S. MCLESTER AND H. C. SHERMAN. J. A. M. A. **118:** 944, 1942. (3) GOLDBERGER, J. AND G. A. WHEELER. Pub. Health Repts. **30:** 3336, 1915. (4) SEBRELL, W. H. AND R. E. BUTLER. Pub. Health Repts. **53:** 2282, 1938. (5) SEBRELL, W. H. AND R. E. BUTLER. Pub. Health Repts. **54:** 2121, 1939. (6) SPIES, T. D., W. B. BEAN AND W. F. ASHE. Ann. Int. Med. **12:** 1830, 1939. (7) KRUSE, H. D., V. P. SYDENSTRICKER, W. H. SEBRELL AND H. M. CLECKLEY. Pub. Health Repts. **55:** 157, 1940. (8) SYDENSTRICKER, V. P., W. H. SEBRELL, H. M. CLECKLEY AND H. D. KRUSE. J. A. M. A. **114:** 2437, 1940. (9) WILLIAMS, R. D., H. L. MASON AND B. F. SMITH. Proc. Staff Meet. Mayo Clinic **14:** 787, 1939. (10) WILLIAMS, R. D., H. L. MASON, R. W. WILDER AND B. F. SMITH. Arch. Int. Med. **66:** 785, 1940. (11) CRANDON, J. H., C. C. LUND AND D. B. DILL. New England J. Med. **223:** 353, 1940. (12) GOODHART, R. S. Report of Committee on Nutrition in Industry, Food and Nutrition Board National Research Council, 1942. (13) DRUMMOND, J. C. Personal communication.

American Institute of Nutrition

Symposium on Fortified Food

April 1, 1942

DEFINITIONS AND STANDARDS OF IDENTITY FOR FOOD

E. M. NELSON, CHAIRMAN

United States Food and Drug Administration

One of the most important provisions of the Federal Food, Drug, and Cosmetic Act of 1938 was the authority to prescribe definitions and standards of identity for foods. The necessity for this provision had been amply demonstrated by experiences in the enforcement of the old pure food law of 1906 which was replaced by the 1938 statute.

While definitions and standards of identity were prescribed in connection with the enforcement of the old law, they were purely advisory. Because they had no legal effect they proved woefully inadequate for the purpose of maintaining the integrity of our food products. The ineffectiveness of the standard for butter led to the enactment of a Federal statute back in 1923 prescribing a definition and standard for this food. This was the only legally binding Federal standard of identity for any food prior to those which have been issued under the new law.

The circumstances which led to the enactment of the butter standard illustrated the need for authority to prescribe standards for foods generally. Butter had been found on the market with a fat content varying from about 65 to 85 per cent or more. Some creameries deliberately worked water into their product, thus obtaining butter prices for water, and at the same time depleting the purchasing power of the consumer dollar in terms of food value received.

The chaotic condition resulting from this cutthroat type of competition led to sponsorship by dairy interests of the bill which became the standard butter law which defined butter as the product ordinarily known as such which is made exclusively from milk or cream or both, with or without common salt, and with or without coloring matter. It prescribed a standard for the content of milk fat of not less than 80 per cent by weight. This legislation was easily enforceable. After its passage, successful court action against butter containing 75 per cent of fat, for example, was easily consummated by testimony of competent analysts showing that the product contained less than 80 per cent fat. The stabilizing effect of the enforcement of this law was of immediate advantage to dairymen and creameries and to distributors and consumers of butter.

This experience brought into striking contrast the inadequacies of the advisory standards. What developed in the manufacturing of fruit preserves is illustrative. The advisory standard for these products prescribed that they be made with the time-honored household formula of a cup of fruit for each cup of sugar, which translated into weight and allowing appropriate tolerances was expressed as "not less than 45 pounds of fruit for each 55 pounds of sugar." But if a manufacturer used only half the required amount of fruit, the Government in the prosecution of the case was required to marshal preserve manufacturers, housewives, and food technicians to testify, not that the standard was violated, but that the product containing so little fruit was not entitled to be sold as a preserve and was not generally recognized as such. It was necessary to repeat this process in each contested case. The results of those actions were by no means uniform, since one jury might give the Government a verdict, while another, with the same facts before it, would render a verdict for the manufacturer. Chaotic conditions developed, with consumers assuming the usual rôle of innocent bystanders.

Obviously it is impossible for Congress itself to assemble and evaluate all of the technical information necessary for the formulation of standards for foods generally. The Act of 1938, therefore, delegated this responsibility to the enforcing agency.

Such delegation of authority gives desirable flexibility to the standards-making process. Standards fixed by legislative bodies could not ordinarily be amended promptly to meet changing conditions in the food industries. New ingredients and processes which represent genuine contributions to the more economical production of our food supply or to the enhancement of its quality are frequently proposed. Certainly it was not the legislative intent that standards should freeze for all time existing practices as to composition and methods of production, and the policy of enforcement officials is very definitely one of avoiding by proper amendments to established standards the hampering of progress in food technology.

The new law provides that "Whenever in the judgment of the Administrator such action will

promote honesty and fair dealing in the interest of consumers, he shall promulgate regulations fixing and establishing for any food, under its common or usual name so far as practicable, a reasonable definition and standard of identity. . . ."

It should be noted that the yardstick laid down for the Administrator's action is that the standard in his judgment shall promote honesty and fair dealing in the interest of consumers. Consumer benefits may flow from a standard in various ways. They may arise from the elimination of practices which debase the quality or nutritional value of the food, and of the use of cheaper substitutes or materials of dubious value.

Standards which follow this expressed purpose of the Act and which have the force and effect of law are invaluable for efficient enforcement and the maintenance of the integrity of the food supply. The importance of these objectives is great in peace-time; in war-time it is even greater. Our experiences in World War One and the experiences England is now undergoing show that with the changes brought about by war-time conditions, such as scarcity of raw materials, rapidly changing price structures, inexperienced factory personnel who replace competent workers transferred to combat services, and unsettled conditions generally lead to greatly increased likelihood of the debasement of our food supply and all that that implies in terms of malnutrition at a time when adequate nutrition of every individual is vitally needed.

It is no contribution to efficiency to consume as dried milk ordinary wheat flour whose creamy tinge results from a coal-tar dye, or to consume as orange juice a synthetic concoction devoid of vitamin C and other nutritional essentials of orange juice. These are but illustrations of many consumer cheats that are now current in England and that arose in our country in the first world war. The existence of reasonable food standards, easily enforceable, is a powerful weapon against the exploitation of the public through such practices.

In the formulation of food standards under the new Act, extensive investigations are required to insure their reasonableness and fairness and that they will genuinely promote consumer welfare. An invaluable background to the consideration of a standard of identity for a particular food commodity is furnished by the files of the Food and Drug Administration, which go back to the beginning of the enforcement of the old law of 1906. These show the development of methods of production, changes in composition which may have occurred, the adulterations which have been practiced, and indicate many of the safeguards needed to protect the public. Usually a nation-wide investigation is made of current practices so that trends can be evaluated and practices which are contrary to consumer interest uncovered. These data are considered by the Food Standards Committee of the Food and Drug Administration, an advisory body, which is composed of two members from that organization and four state officials connected with the enforcement of state food and drug laws. Usually one or more informal conferences are held by the Committee with representatives of the affected industry and the consuming public. The recommendations of the Standards Committee are considered by the Food and Drug Administration. The Administration then refers its own proposals to the Administrator of the Federal Security Agency for consideration and the annouueement of a formal hearing under the provisions of section 701(e) of the Act. This section requires that a proposal in general terms be published with notice of public hearing thereon to be held not less than thirty days after the date of the publication. The hearing is usually attended by a large number of manufacturers from the affected industry, by commercial interests supplying materials to this industry, and by representatives of consumer groups. While the hearings do not have the same degree of formality as court proceedings, they are orderly and a record is kept of all testimony. The records of food standard hearings so far held are replete with factual information concerning all phases of the manufacture and distribution of the products, of the potentialities for abuses, and of needed changes in existing practices.

After completion of the hearing, the testimony is thoroughly studied and a tentative definition and standard, based exclusively on the recorded evidence, is formulated and published by the Administrator, with invitations to all interested parties to submit written comment upon the proposed standard within a specified time. Upon a review of this written comment a final definition and standard of identity is published by the Administrator. Under the terms of the Act this cannot become effective in less than ninety days from the date of its publication, unless an emergency of some kind exists. Provision is made for review by circuit courts of appeal. After the standard becomes effective, any food which purports to be or is represented as a food for which a standard has been fixed is misbranded and therefore liable to proceedings under the statute unless it conforms to all the requirements of the standard.

Of particular interest among the various standards that have so far been promulgated or are in process of promulgation have been the nutritional problems arising in connection with evaporated milk, oleomargarine, enriched flour, and enriched bread. In each of these instances the addition

of one or more vitamins or minerals was proposed. In the case of evaporated milk and oleomargarine there existed at the time the hearings were held well established practices of fortifying the articles with added vitamins.

The final standard for evaporated milk authorized fortification with vitamin D, with appropriate label declaration, in a quantity not less than 7.5 United States Pharmacopoeial Units per avoidupois ounce, which is the approximate equivalent of 135 units per quart for fluid milk. The final standard for oleomargarine authorized the use of vitamin A, added as fish liver oil or as a concentrate of vitamin A from fish liver oil, with any accompanying vitamin D and with or without vitamin D concentrate. If any such addition is made it must be in such quantity that the finished oleomargarine contains not less than 9,000 U.S.P. units of vitamin A per pound. The testimony upon which these additions were authorized clearly showed need for such fortified articles by many persons, and that such additions would promote the interests of these consumers.

Prior to the hearing on the proposed standards for flour, the practice of fortification with vitamins or minerals was such that the kinds and quantities of vitamins and minerals added varied widely between individual firms. The diversity of vitamins and minerals proposed as optional additions to flour and the lack of any rational plan of enriching flour were responsible for the adjournment of the hearing for more than a month to permit a more deliberate consideration of the problem. When the hearing was resumed, testimony was given to show that the diets of persons in various sections and in various income groups do not furnish sufficient amounts of certain necessary nutritional elements to maintain health; that the deficiencies are more extensive and more prevalent in cases of persons with low incomes; and that such persons usually acquire a large part of their energy requirements from flour because of its cheapness as a source of energy. This and related evidence made it possible to formulate an approach to the problem of enriching flour and perhaps food products generally.

Following the statutory requirement for the promotion of honesty and fair dealing in the interest of consumers, it was concluded that enrichment with vitamins and minerals should be authorized only under conditions which in substance are as follows: First, the existence in a substantial portion of the population of dietary deficiencies in specified nutritional elements should be shown; second, the regular consumption in substantial quantities of the food under consideration in the normal dietary of those persons should be demonstrated; third, the suitability of that food should be established as a carrier to insure that the needed nutritional factor will reach the consumer (for example, a proposal that flour be enriched with vitamin C was abandoned under this concept); fourth, the availability of supplies of the needed nutritional factors should be shown at costs permitting the use of nutritionally significant quantities without materially increasing the price of the food and thus making it less available to the groups having greatest need for enrichment.

Considerable controversy arose concerning the question of whether the indiscriminate and miscellaneous enrichment with one or two items which had been indulged in by some manufacturers should be continued. However, the record clearly pointed to the conclusion that indiscriminate enrichment of foods with vitamins and minerals would tend to confuse and mislead consumers, would give rise to confused claims regarding the beneficial effects of such substances; that it would be likely to lead to the impression on the part of some consumers that a single food with an added vitamin or mineral would meet all nutritional needs, and that the limited enrichment of selected staple foods would best promote consumer understanding of the relative values of enriched and natural foods and make more likely the selection by consumers generally of diets which are nutritionally adequate.

It was out of these concepts that the final standard for enriched flour was issued, prescribing minimum and maximum levels for vitamin B_1, riboflavin, nicotinic acid, and iron with the optional use of prescribed quantities of vitamin D and calcium. The testimony at the hearing indicated that supplies of riboflavin would be available by the time the standard could become effective. This did not prove to be the case, and the requirement with respect to riboflavin has been postponed.

The same consideratons applicable to enriched flour are applicable to enriched bread. Hearings were held some time ago on enriched bread, but no standard has as yet been issued. It is expected that one will be published within the near future.

These are only a few instances which illustrate the utility of definitions and standards of identity for foods in safeguarding the public interest and in maintaining the integrity of our food supply. While the examples of enriched flour and enriched bread are unique in that they apply to articles which did not exist a short time ago, these illustrate the flexibility of the standards-making provision and its usefulness in protecting the public against exploitation by programs dictated more by commercial motives than by public interest.

FOOD CONSUMPTION STUDIES AND DIETARY RECOMMENDATIONS

HAZEL K. STIEBELING

United States Department of Agriculture

We in this country are fortunate in the great variety and abundance of our food resources. But if our food supplies, present and potential, are to serve us best from the standpoint of nutrition, there is need for better utilization and a more equitable distribution.

The latest information regarding the distribution of food and nutrients among various population groups comes from Federal studies of family food consumption made in 1936. Current studies are under way. The facts which emerge are fundamental to a discussion of the problem of fortification or enrichment of food.

If we would equitably ration all of our food supplies and if then every person ate the kind of meals that were in accord with his nutrition needs rather than his food habits, we could have diets that would about meet the National Research Council's recommendations of May 1941 except for riboflavin. But left to ourselves we don't achieve any such dietary levels. Some of us want to eat what we like—whether it provides for good nutrition or not. Some of us can't afford to buy the food we know we need, even though we would like to. But many more than now do, could have nutritionally good meals, if everyone knew what he needed and knew how to get the most for his food expenditures, and if everyone really believed good nutrition to be worth the effort it takes to plan for adequate diets and learned to like the foods that this would require.

We found, in 1936, comparatively little difference from one income class to another in quantities consumed per capita of grain products, fats, and, in villages and cities, of sugar. On farms there appeared to be some increase in sugar consumption as incomes rose. In villages and cities, and on farms as well, there were moderate increases in the consumption of milk, eggs, and meat at successively higher income levels, with the rate of increase about the same for each of these three groups of products. Of fruit and vegetables (other than potatoes and dried beans and peas) there was a marked increase in the quantities consumed as incomes went up. In general, the rate of increase in the consumption with successively higher incomes was greater in the lower ranges of the income scale—e.g., under $1,500 a year—than in the upper.

As would be expected from such trends, diets of the higher income groups tend to provide more protein, minerals, and vitamins than those of low-income groups, both absolutely, and relative to caloric value. Of the seven nutrients considered (protein, calcium, iron, vitamin A value, thiamine, riboflavin, and ascorbic acid), diets of low- and high-income groups differed most with respect to calcium, vitamin A, ascorbic acid, and riboflavin, though there are, naturally, wide differences in the richness of diets with respect to various nutrients, even among those having food of the same money value.

Our studies indicate that fewer than one-fifth of families in this country had food supplies in 1936 that met the National Research Council's recommendations with respect to the seven nutrients studied. About a fourth of the farm families were in this category, but only about one-seventh of the nonfarm.

The proportion of families attaining the levels specified with respect to *individual* nutrients was of course higher than the proportion meeting the recommendations in *all* respects. Nutrients are so distributed in common foods that meals may easily supply plenty of some but relatively little of others. For example, a "double" orange juice for breakfast would safeguard the day's vitamin C quota, but do little for the calcium and riboflavin. A quart of milk a day would provide the needed margin of the latter two, but not the former.

Our 1936 studies suggest that at least 60 per cent of the village and city families fell short of the National Research Council's dietary recommendations with respect to calcium, thiamine, ascorbic acid, and/or riboflavin, and 30 per cent with respect to vitamin A. On farms the picture is brighter. But even there, a quarter fell short of the recommended dietary goals in calcium and/or vitamin A, and half or more with respect to thiamine, riboflavin, and/or ascorbic acid.

We have no data as to the extent of undernutrition or malnutrition in the population studied. The fact that a family fell short of meeting the dietary goals does not necessarily mean malnutrition. Undoubtedly the recommendations for various nutrients differ in the margin they provide over average minimum requirements; for some nutrients, of course, requirements for optimal nutrition are far higher than requirements for maintaining an average state of well-being.

Data from our studies represent consumption in the economic sense, rather than physiological intake. Our studies throw no light on how the food supply of a family was distributed among its members, or of the amount of edible food wastes in the kitchen or on the table, or of the invisible losses in food preparation; we deducted only

average quantities of inedible refuse from purchases when computing nutritive value. In contrast, the dietary recommendations of the National Research Council's committee with which we here compare the nutritive values of the food supplies, represent actual intake and do not make allowances for extensive losses in cooking. Hence, all of our figures on dietary adequacy as well as those showing the proportion of families meeting the committee's recommendations tend to be optimistic. For example, because of losses in food preparation, figures from our studies undoubtedly are high as compared with actual intake, especially for thiamine, riboflavin, and ascorbic acid. And our figures for vitamin A err on the high side, too, since 60 per cent or more of the

TABLE 1

Quantities of major food groups delivered to the Nation's kitchen per capita in 1936, as estimated from two types of source material

FOOD GROUP	UNIT	CONSUMPTION AS ESTIMATED FROM—	
		Statistics on production, stocks, etc.	Family food records
Fluid milk (or other forms equivalent in nonfat solids)	qt.	173	175
Vegetables and fruit	lb.	495	519
Potatoes, sweetpotatoes	lb.	136	148
Dry beans, peas, nuts	do	11	13
Tomatoes, citrus fruit	do	86*	81*
Leafy, green, yellow vegetables	do	65*	84*
Other	do	197*	193*
Eggs	doz.	22	22
Meat, poultry and fish	lb.	134	119
Flour and other cereal products	do	197	208
Butter and other fats	do	66	58
Sugars, sirups, preserves	do	74	76

* Urban only; all other figures, national.

vitamin A value of family diets tend to be derived directly from plant sources, whereas the Committee's specifications were based on the premise that a large proportion would be derived from preformed vitamin A.

We believe that our data on the quantity of food delivered to the Nation's kitchens to be fairly reliable, however. When we take our figures derived from family food records obtained from communities in various regions, at different seasons, for groups differing in race, income, and household composition, and weight them according to the importance of each group in the total population (as estimated from data from many sources), the resulting data are in substantial agreement with another independent estimate of the per capita quantities of food delivered to the Nation's kitchens. The latter, made by O. V. Wells of the Bureau of Agricultural Economics, is based on the Department's statistics of production, imports, exports, and changes in inventories of food on hand, with deductions made by Mr. Wells for waste in processing and marketing as well as deductions for feed, seed, and industrial uses.

The two sets of figures agree within 5 per cent for milk, vegetables and fruit as a group, eggs, flour and cereal products, and sugars. The greatest discrepancies (exceeding 10 per cent) are for fats and meats on the one hand (for these, data from family consumption studies are the lower of the two) and for dry beans, peas and nuts and for leafy, green and yellow vegetables on the other (for these, data from family consumption figures are the higher). The agreement between the two sets of figures, independently derived, is remarkable, considering the difficulties involved in preparing each.

Because of the widespread interest in these figures, table 1 is included to compare the two estimates of consumption of major groups of food in 1936.

Over 60 per cent of the calories in 1936 were derived from grain products, fats, and sugars—most of which were in highly refined forms. Two thirds or more of the vitamins, minerals, and proteins were derived from vegetables, fruit, milk, eggs and meat—all of which together accounted for less than 40 per cent of the calories. These classes of food are rich sources of these nutrients, but the total quantities consumed fall short of the amounts needed to provide the kind of meals that the National Research Council's Committee recommends.

If everyone in this country were to modify market lists just enough to meet these recommendations—whether food budgets are large or small—there would be a tremendous shift in market demands. Much more milk and cheese—probably at least 50 per cent more—would be consumed. These products are unusually economical food sources of both calcium and riboflavin. There would be demand for twice the quantity of leafy, green, and yellow vegetables, considerably more of vitamin C-rich fruits, as well as a larger share of our grain products in lightly milled or enriched forms. More eggs and meat than many low-income groups now eat would be called for.

Due to a combination of factors some progress toward better nutrition in this country has been achieved since 1936. In 1940–41 national per capita consumption of fresh vegetables was 12 to 14 per cent higher than 5 years earlier; of canned vegetables, 5 to 8 per cent; of citrus fruit, 30 to 40 per cent; of other fruit, 10 to 15 per cent; of

meat and eggs, 8 to 10 per cent; though the increase in much needed dairy products was less than 5 per cent.

The consumption of grain products decreased slightly during this period, but something new was added. Upon the recommendation of the National Research Council, millers and bakers began in 1941 to put on the market white flours and breads "enriched" with iron, thiamine, and nicotinic acid. (After July 1, 1942, these products will also be enriched with riboflavin.) Since these nutrients are among those in which diets of low-income groups often are deficient and since bread is an important article of diet among such groups, this step automatically improved the diets of all of those white-bread users who read the labels and chose the enriched product.

The foods of which consumption was higher in 1940–41 than 5 years earlier were, in general, those most in demand when purchasing power is increased. Compared with 5 years earlier, many families had higher incomes in 1940–41 and could buy more and better food. Food prices were at a relatively low level in 1940 and early 1941, so that despite the rises in the cost of food during the latter part of 1941, incomes of wage earners bought more food both in 1940 and 1941 than in 1936. Indeed, it seems possible that with their increased power to buy, as many as one family out of every seven that had poor diets in 1936 was able to obtain a fair or good diet in 1941.

I think we should remember, however, that some families probably were worse off nutritionally in 1940–41 than five years before. These were the low-income nonrelief families whose economic status did not improve with the general upward trend in business and who did not benefit from public food distribution programs. Some have been caught between the upper millstone of a fixed low income and the lower stone of rising food prices. This is a danger point to which we must look.

Many families on relief fared better in 1940–41 than 5 years earlier because the Department of Agriculture's several food distribution programs helped to channel a larger-than-usual share of the food supply to low-income groups. This was done through the food stamp plan, direct distribution of surplus commodities through welfare agencies, school lunches, and arrangements for low-priced milk. These programs were developed in the middle 1930's and later—to broaden the farmers' markets for food and to improve dietary levels of low-income families.

All of the facts at hand indicate that by participating in these programs families got better balanced as well as more abundant diets. The school lunch and food stamp program reinforced diets in many directions in 1940, but especially with respect to vitamin A, thiamine, and ascorbic acid. Although the food obtained directly through these programs contributed but little to correcting the frequently recurring shortages of calcium and riboflavin, the mere fact that the programs were in operation helped to this end: that stamp plan participants buy more of the much needed milk than comparable nonparticipants. Furthermore, sponsors of school lunches in many communities provided milk to supplement meals that could be prepared from foods furnished by the Surplus Marketing Administration.

Two government-aided programs are now in operation that directly encourage milk consumption—"Penny-Milk" for school children and low-priced milk for family groups. That families will greatly increase their purchases of milk when it is available at a low price is illustrated by figures from a study made in Washington, D. C. Before the program was begun white participants used the equivalent (in nonfat milk solids) of 2½ quarts of fluid milk per person per week. They used 4 quarts after the program went into effect. Negro families consumed the equivalent of less than 2 quarts per person a week during pre-program study, compared with about 3½ quarts afterward.

Altogether the various food distribution programs brought almost 175 million dollars worth of additional food to an average of more than 11 million underprivileged persons in 1940–41. This amount is small compared to the total number in need of more food and better diets. The diets (and presumably the nutritional health and working efficiency) of still more low-income families could be improved were sufficient funds available for subsidizing food consumption.

Since enough food is available only if enough is produced, 1941 is to me to be a special year in the history of nutrition in this country. More food was produced than ever before, and for the first time goals for agricultural production were planned that covered the entire range of food products. Also, for the first time in such planning, human nutritional needs as well as probable effective market demand were consciously considered in setting the goals. This is a tremendously significant precedent.

The 1942 goals were not and, of course, could not be determined solely by needs for optimal nutrition. Full food production must be paralleled by two other circumstances:

1. Families in cities and villages must have the resources to buy the food they need. Nothing is more discouraging to the producer than to find no buyer.
2. People everywhere must come to know what they need for good nutrition and to *want*

well-rounded meals that supply all the nutrients needed in good proportions.

By no means are all of the poor diets of this country found among low-income groups. There is room for considerable improvement in diets at all income levels—among families who live on "Main Street" as well as among those "across the tracks." In villages and on farms, many families, could have far better diets without any greater outlay of cash by more and better home growing of the family's food supply. Wiser choice when buying and a better job of cooking to conserve food values would yield returns in better nutrition. To all these we hope the current educational programs reinforced by the National Nutrition campaign will make increasingly significant contributions.

Despite measurable progress from 1936 to 1941, and a current dietary level that is far above that in most parts of the world, we all realize that there still is wide disparity between present consumption habits and what the science of nutrition would recommend. Changing dietary habits through educational processes is a slow business. The economic obstacles to improved diets are many. Hence it is appropriate that the Institute of Nutrition should sponsor a symposium on fortification of foods—to consider steps needed to improve the nutritive qualities of foods on the market and steps needed to protect the consumer.

Not unmindful of the importance as a public health measure of appropriately enriching certain basic foods,—e.g., milk with vitamin D; bread and flour with the B-vitamins; oleomargarine with vitamin A; salt with iodine—I believe that our long-time program should concern itself with helping to break down social and economic barriers to better diets. We should preserve the characteristic nutritive qualities of food, enriched to modest levels if it seems expedient, but only in such a way that *variety* shall continue to be a desirable quality of diets. Until we know much more than we now do about human nutritional needs, we would do well to depend upon natural products rather than synthetics for the greatest share of our nourishment.

CLINICAL OBSERVATIONS BEARING ON FOOD REQUIREMENTS

EDGAR S. GORDON

Departments of Physiological Chemistry and Medicine, University of Wisconsin, Madison

Present concepts of the clinical manifestations of nutritional deficiency disease, the daily requirements for humans of the various nutrients, and to some extent, the physiological functions of the different food factors, have evolved through the combined efforts of students of nutrition, many of whom have worked on clinical problems. Gradually, over the past decade the foundations for the science of human nutrition have been laid in an ever growing mass of biochemical and physiological data which is constantly being improved as more accurate techniques and quantitative methods are devised. It is the purpose of this review to summarize a few of the many excellent studies in the field of clinical nutrition which have added so much to our present knowledge of the food requirements of man and the incidence and nature of human deficiency diseases.

VITAMIN A. Avitaminosis A is now recognized as a very different entity from the descriptions of xerophthalmia given in the older text-books. Its manifestations are varied, and even mild grades of the disorder often produce recognizable symptoms and signs. The function of vitamin A in the visual cycle has been so abundantly demonstrated that its importance can hardly be questioned. The application of various instruments for the measurement of the speed of dark adaptation, as an index of vitamin A nutrition, however, is still an unsettled question. Failure of general acceptance of this principle as a valid diagnostic aid probably arises from the realization that vitamin A is only one of the constituents of the chemical "systems" necessary for the synthesis of visual purple. A number of enzymes, an adequate supply of carbohydrate, other vitamins, a protein, and perhaps even intact adrenal cortical function are also known to be necessary, so that the adaptometer may measure a weakness at only one link in a chain of chemical reactions. In general, a fairly good agreement between biophotometer and plasma vitamin A levels is recognized, although many dissenting reports have appeared in the literature. Particularly under dispute is the validity of the rate of adaptation as a measure of very early, sub-clinical avitaminosis A. Since the rod reponse is of greater magnitude than that of the cones, a special adaptometer has been devised for measuring the rate of adaptation in the former. In spite of this, the function of both rods and cones is impaired in vitamin A deficiency to an approximately equal extent, and estimation of this impairment in either case should be equally satisfactory. Of the

many valid objections which have been raised against these visual techniques grouped as a whole, the importance of refractive errors and the necessity for good cooperation on the part of the subject are probably the most important. The total objectivity of blood analysis is lacking.

Unusual interest has been aroused by the observations of Kruse (1) that certain gross and microscopic changes in the external structures of the eye may act as reliable criteria of vitamin A deficiency. These lesions, xerosis conjunctivae and Bitot's spots have been recognized for 80 years but their significance has been considerably clarified by Kruse's studies with the biomicroscope. The actual details of the changes need not be reviewed here. Doubt and disagreement as to the etiology of these changes are still quite widespread, particularly among ophthalmologists. This particular group is especially critical of the avitaminosis theory for the etiology of pinguecula, a lesion which Kruse believes is a different stage in the same process that produces Bitot's spots (2). The remarkably high incidence of conjunctival lesions of this general type, especially in individuals of middle age or past makes the establishment of correct etiology of more than passing interest. Whether or not complete agreement on this point is eventually attained, this diagnostic method may be criticized as lacking quantitative significance, except in a rough subjective way. Chemical study of blood possesses a definite advantage for that reason.

Experimental induced vitamin A deficiency in adults has been studied by Brenner and Roberts (3). Evidences of avitaminosis tested were changes in visual adaptation, abnormalities in the visual fields, histological changes in the skin and conjunctivae, changes in the total and differential blood counts and fluctuations in blood vitamin A and carotene. After seven and one-half months on the diet, no changes could be observed except for a low blood carotene. These findings raise some interesting questions concerning the speed of appearance of so-called "early changes" during naturally occurring avitaminosis A, and suggest that multiple deficiencies again may provide the explanation.

A great many studies have demonstrated a clear-cut quantitative relationship between blood vitamin A content and dietary intake, both in experimental animals and in humans. Many of these same investigations have also emphasized the importance of the liver, not only as the most important storage depot for vitamin A, but also as the site of enzymic splitting of carotene into the vitamin. Ralli, Bauman and Roberts (4) found consistently low plasma levels of both vitamin A and carotene in patients with cirrhosis of the liver, correlated with poor visual adaptation. The probable explanation for these findings was an impairment of conversion of the provitamin associated with extensive liver damage. Ralli, Papper, Paley and Bauman (5) corroborated this hypothesis by demonstrating a low liver storage capacity in this disease. A lesser degree of the same change was observed in acute infections. These observations have been widely substantiated in general clinical experience with blood vitamin A determinations in a variety of conditions. It may be stated with assurance, therefore, that all subjects with serious liver disease may be expected to have clinical avitaminosis A due to the combined effects of three factors; 1, low storage capacity; 2, poor absorption of fatty materials from the gastro-intestinal tract, and 3, defective conversion of the provitamin. Abels, Gorham, Pack and Rhoads (6) have reported a similar metabolic defect in many patients with malignant disease which causes a low level of vitamin A in blood plasma. No changes occur in the figures for carotene. Administration of yeast, lipocaiac or choline produce large, significant rises in the blood vitamin A values, probably ascribable to the lipotropic action of those substances. Surgical removal of the cancer also effects this same change.

THIAMINE. The clinical diagnosis and laboratory recognition of thiamine deficiency have received an immense amount of attention recently, in an effort to clarify the diagnostic criteria and to establish the daily human requirement. In a series of papers Williams and Mason (7) have reported the results of investigations of a group of experimentally induced thiamine deficient subjects. All other nutrients were carefully supplied in order to avoid complicating deficiencies. Objective manifestations did not appear for many weeks, but the urinary excretion of thiamine fell to 15 to 30 gamma daily within 15 days. The subjects lost their appetites and many developed nausea and vomiting. They became depressed, irritable, quarrelsome, inefficient and forgetful. Several lost their previous manual dexterity and became confused and inattentive to details. Electrocardiographic changes were noted in only 3 individuals, and other laboratory data were not particularly striking. No rise in the bisulfite binding substance of blood serum was noted.

Urinary studies demonstrated a very satisfactory correlation between dietary intake and urinary excretion. By means of a test dose of 1 milligram administered subcutaneously followed by urine collection for the subsequent hour, a sharp differentiation between deficient and normal subjects was possible. In the former, excretion amounted to from 2 to 12 per cent of the test dose, the exact percentage depending upon the dietary content of thiamine. In the latter, excretion was

consistently above 15 per cent and usually between 20 and 45 per cent. From these results the authors concluded that a basic thiamine excretion of 100 micrograms or more daily and the urinary excretion of 20 per cent or more of a test dose given under the above specified conditions, constituted dependable evidence of normal nutrition relative to thiamine. They also concluded that the daily human requirement amounted to not less than 0.5 mgm. nor more than 1.0 mgm. per 1000 calories of food.

The excretion figures of Williams and Mason, in general, are in accord with those of Robinson, Melnick and Field (8), who used a different assay method for the vitamin. The latter authors found all well nourished male subjects to excrete more than 90 micrograms per 24 hours and all females 60 micrograms or more. Deficient males excreted 66 micrograms or less and females 43 micrograms or less. The excretion of all deficient subjects was 7 per cent or less of the test dose of 5 milligrams given orally followed by a 24 hour urine collection. Thus a clean-cut separation of normal from abnormal cases was possible, and a sex difference became apparent, due perhaps to a corresponding difference in total food intake. In a subsequent paper (9) these same authors reported no change in bisulfite binding substance of blood after 22 days on a thiamine deficient diet.

In a study of a single case, a 60 year old woman Elsom, Lukens, Montgomery and Jonas (10) reported the beginning appearance of deficiency symptoms after 5 weeks on the experimental diet, which contained sub-optimal amounts of the entire B complex group. Urinary excretion of the various missing components was not studied, but attention was directed to the metabolic deviations from normal. Blood sugar, following the administration of glucose by mouth, failed to return to normal at the end of 4 hours, during the deficiency period. Blood pyruvic acid following glucose administration also failed to return to normal, but the fasting values continued within normal limits throughout the experiment. Bisulfite binding substance values for blood were not altered. Neither thiamine alone, nor thiamine plus riboflavin corrected these abnormalities, but administration of yeast was completely effective.

In accord with most of these findings are those of Shils, Day and McCollum (11) who likewise were not able to demonstrate any rise in blood bisulfite binding substance in two normal men and two normal women, who maintained themselves for periods up to 37 days on a diet deficient in thiamine only. Although thiamine excretion fell to an extremely low level, no measurable disturbance in carbohydrate metabolism occurred, and no symptoms attributable to lack of thiamine appeared at any time during the experiment.

These findings are in sharp contrast to those of Banerji and Harris (12), who found an increase in urinary bisulfite binding substance to 3 or 4 times normal within one week after the initiation of a beri-beri producing diet. This occurred in spite of a continued urinary excretion of thiamine at a moderately high level. Jolliffe, Goodhart, Gennis and Cline (13) observed subjective symptoms in 4 out of 5 individuals on the fourth day of a thiamine low diet. Objective manifestations appeared as early as the fifth day and consisted of skin hyperesthesia, electrocardiographic changes and calf muscle tenderness. A sharp fall in the urinary output of thamine occurred within 24 to 48 hours after the deficient diet was initiated. The values for the deficiency period vary between 35 and 105 micrograms per day, constituting roughly 15 to 20 per cent of the excretion during the control period, and represent from 7.4 to 23 per cent of the estimated intake.

Thus, it is apparent that studies on human subjects, even when conditions are rigidly controlled, show certain fundamental inconsistencies which have not yet been resolved. The discrepancies between blood and urine bisulfite binding substance values suggest that the renal threshold for these substances may be so low that a rise in urinary excretion represents the earliest measurable change. The greater portion of available evidence indicates, at least, that fasting blood bisulfite binding substance values are not changed even by prolonged sub-optimal intake of thiamine. There is, however, moderately abundant evidence that an alteration in both the blood glucose and pyruvate curves following ingestion of glucose may take place early in thiamine deficiency, thus constituting a possible diagnostic measure for subclinical or borderline conditions. Robinson, Shelton and Smith (14) and Bueding et al. (15) have made diagnostic use of this technique, but the reliability of this procedure can be questioned.

RIBOFLAVIN. Considerable recent progress has been made in defining the biochemical rôle of riboflavin in intermediary metabolism, but our knowledge of the clinical picture of ariboflavinosis has remained essentially unchanged for several years. It is becoming increasingly clear to most observers that the naturally occurring deficiency lesions are nearly always of multiple etiology. For this reason pure, uncomplicated ariboflavinosis in humans is virtually an unknown entity. Its experimental production may yield much helpful information.

Experimental human riboflavin deficiency has been produced by Williams and Mason (16) by a diet supplemented by all other nutrients. Urinary excretion of riboflavin fell progressively throughout the period of depletion, but at the end of six months no demonstrable changes had taken

place in the subjects from either the subjective or objective standpoints.

The ocular lesions of riboflavin deficiency are now well known, and their etiological relationship to this vitamin has withstood the test of continued observation in a more satisfactory manner than have the older and perhaps better known lesions of cheilosis. The latter were healed by the administration of pyridoxine by Smith and Martin (17) and more recently Machella (18) has shown that even nicotinic acid and ascorbic acid would produce a satisfactory response, from which the author concluded that cheilosis is probably a multiple deficiency lesion.

Both chemical and microbiological assay methods are available, the results of which check very well. By means of quantitative studies, the daily human requirement of about 2.5 milligrams has been established (19). The actual incidence of ariboflavinosis is variable in different communities, depending probably upon the dietary habits of the population. Thus it seems almost certain that this condition is encountered with much less frequency in many Wisconsin clinics, in the center of a dairy region, than in many other areas. The coexistence of ariboflavinosis in most pellagrins has been repeatedly emphasized. Further large scale studies of urinary excretion of riboflavin would probably be helpful in broadening the general perspective of deficiency disease in non-endemic pellagra areas.

Nicotinic acid. Clinical studies of nicotinic acid have been seriously delayed by the unavailability of a satisfactory analytical method to apply either to blood or urine. Measurement of coenzymes I and II in blood appeared at first as a possible solution to the problem, but improvement in methods has now made it clear that blood coenzyme values do not fluctuate significantly in pellagra (20, 21). Tissue coenzyme content diminishes during nicotinic acid deficiency but tissues are inaccessible for diagnostic purposes. Measurement of the urinary excretion of nicotinic acid is complicated by the multiple pyridine compounds present in the urine, which represent various excretory forms of the vitamin. Chief among these are nicotinuric acid and trigonelline. Using the cyanogen bromide and aniline method, Sarett, Huff and Perlzweig (22) have developed a preliminary procedure for measuring and allowing for the interfering substances. They found an average daily excretion of 0.7 to 2.2 mgm. of nicotinic acid, and about 15 milligrams of trigonelline in 33 healthy medical students. These figures compare reasonably well with the 3.0 to 5.0 mgm. daily excretion of nicotinic acid reported by Patton, Sutton and Youmans (23). Field, Melnick, Robinson and Wilkinson (24), after a thorough exploration of this chemical method found no correlation between blood nicotinic acid values and clinical status, and concluded that this technique applied to blood or urine was useless as a diagnostic test.

The hope that measurement of a urinary porphyrin in pellagrins might be developed into a diagnostic method has been destroyed by the demonstration by Watson (25) and Kark and Meiklejohn (26, 27) that the substance originally measured by Beckh, Ellinger and Spies (28) is in reality the indol derivative, urorosein. Ether soluble porphyrin actually does appear in the urine of pellagrins at times, but it clearly does not bear a dependable relationship to the deficiency state.

Thus recognition of sub-clinical nicotinic acid deficiency is still dependent upon routine clinical observation and its true incidence is thereby made extremely difficult to estimate. Further exploration of this important field will proceed only as fast as analytical methods can be developed and applied.

Ascorbic acid. The function of ascorbic acid in human nutrition has now been sufficiently clarified to permit an intelligent understanding of deficiency states, but there is still a striking lack of information available concerning the mechanism of action of this substance in intermediary metabolism. The excellent study of Crandon, Lund and Dill (29) has provided a helpful new perspective on the entire problem of clinical scurvy. In this experimentally induced and exhaustively studied human case, all coexisting, interfering deficiencies were carefully avoided so that the data are correctly representative of pure uncomplicated scurvy. It is of interest to note that the first objective manifestations appeared on the 134th day of the experiment after the plasma ascorbic acid level had been at zero for 93 days. During the first half of the deficiency period the ascorbic acid content of the platelet and white cell layer of blood gave an accurate index of the rate of depletion while the plasma analyses were satisfactory for less than half that time. No changes in the gums were noted at any time and the Rumpel-Leeds test, so often considered to be diagnostic of scurvy, remained negative throughout. The typical perifollicular hemorrhages in the skin first appeared on the 161st day of the diet, in sharp contrast to the authentic reports of 18th century sailors who often developed the full blown or even fatal disease in from 60 to 120 days. Multiple coexisting deficiencies may perhaps explain these differences between naturally occuring and experimental scurvy.

The dependability of plasma ascorbic acid estimations as an index of the dietary intake of that substance has been further studied by Bryan et al. (30) who have reported an excellent degree of

correlation between these two variables, sufficient to justify considerable confidence in the clinical use of plasma determinations in the study of individuals with sub-optimal intakes, as well as borderline and early deficiency states. It is clear, however, that in extreme malnutrition and in clinical scurvy, the ascorbic acid content of the white cell layer is likely to be a more valuable diagnostic guide.

The factors which influence the distribution of ascorbic acid between red cells, white cells, platelets and plasma have been thoroughly studied by Heineman (31) and by Butler and Cushman (32). Their reports have reemphasized the greater significance of either plasma or whole blood determinations in evaluating degrees of saturation as contrasted to the importance in serious deficiencies of the content of the white cell and platelet layer which "provides an index of ascorbic acid deficiency that extends beyond the limits defined by plasma values."

Routine hospital experience with plasma ascorbic acid studies in a wide variety of clinical conditions almost invariably reveals a high incidence of sub-optimal concentrations, (using 0.8 or above as optimal levels) although values of zero are encountered much less frequently. The meaning of chronic sub-optimal ascorbic acid intake, however, still remains to be defined in terms of objective laboratory evidence of impaired structure or function. The recent interesting paper by Feller, Roberts, Ralli and Francis (33) is an excellent example of the attention which is being focused upon this problem. In this investigation the authors were completely unable to define severe ascorbic acid and vitamin A deficiency in humans in terms of altered immunological reactions. Antibodies in nasal secretions and blood, complement titer and phagocytic activity all remained unchanged throughout the experiment. These findings call for a critical re-examination of the evidence which constitutes the basis for our present accepted ideas of disability resulting from nutritional deficiency disease.

The question of the daily human requirement of ascorbic acid must be considered as still unsettled. The clinical study of Ralli, Friedman and Sherry (34) demonstrated that plasma levels of 1.0 mgm. per cent could not be maintained in three healthy male adults unless the daily intake was set at about 100 mgm. They suggest this figure as the optimum daily intake. Crandon et al. (29) on the other hand estimated the daily utilization at between 30 and 45 mgm. which may be taken as a minimum rather than an optimum figure. Storvick and Hauck (35) found that daily intakes of 75 to 160 mgm. were necessary in six subjects, in order to maintain tissue saturation. Estimates of the daily requirement therefore vary from 50 to well over 100 mgm. according to the criteria of adequate intake employed in the various studies. One naturally wonders, under these circumstances whether the optimal plasma level of 1.0 mgm. per cent is not too high. May it not be advisable to set this level at 0.8 or even 0.6 mgm. per cent, or is complete tissue saturation necessary for optimal nutrition? These questions may be difficult to answer, but the incidence of latent ascorbic acid deficiency in the population as a whole depends upon that answer.

IRON. Interest in the metabolism of iron has been stimulated by the use of radioactive isotopes and other techniques of study. The minimum daily requirements for humans have undergone a steady revision downward during the last decade with a realization that a healthy adult male requires almost no iron intake since there is no appreciable loss either in urine or feces. The required dietary allotment would therefore not exceed a few micrograms daily, and so far as iron balance is concerned, anemia must be explainable on the basis of blood loss (36). In females the slight but constant loss of iron in the menstrual flow must be balanced by appreciable intake, which, however, has recently been shown to be much smaller than had previously been estimated. Leverton and Marsh (37) showed that young women receiving from 10.44 to 7.21 mgm. of iron daily in the diet stored from 1.3 to 1.5 mgm. daily, which was more than enough to replace the loss of normal menstruation. A strong positive balance cannot be maintained indefinitely, however, but must be altered by a lower rate of retention or some other mechanism, as soon as the available storage capacity has been filled. In general, it seems probable that the well known 15 mgm. daily requirement for adult humans is higher than is really necessary, provided blood loss need not be considered.

That the storage capacity of humans is large is suggested by the careful studies of McCance and Widdowson (38) who were able to recover from urine and feces only 1.4 per cent of injected iron in healthy adults, indicating probable storage of the remainder. They were unable to show any appreciable excretion of iron into the intestine. The results of these studies indicate that the ultimate control over iron balance is exerted through absorption or failure of absorption from the gastro-intestinal tract depending upon the metabolic needs at any particular time. Moore et al. (39) found this absorption to be most efficient with ferrous iron, but the presence of a reducing agent such as ascorbic acid facilitated the absorption of even ferric iron. These findings were believed to indicate that only the ferrous form is absorbed. Medicinal iron in this form is

very effective in the treatment of anemia, approximately 25 mgm. of iron per day being necessary for a rise of 1 per cent in hemoglobin. At this rate, 50 days would be required for a rise of 10 per cent in hemoglobin (36).

Through observation of a transitory peak of hemoglobin regeneration after 60 days of iron administration, in both normal and anemic subjects, Fowler and Barer (40) believe they have evidence that iron has an additional stimulating effect on hemoglobin formation over the simple replacement of depleted iron reserves. There has been no further substantiation of this theory.

There have been many surveys of hemoglobin values in representative groups of otherwise normal people, which in general confirm the impression of physicians in large clinics that mild anemia from iron deficiency (blood loss) is extremely common. Indeed it is probably the most common deficiency disease.

Unfortunately time does not permit even a brief discussion of a number of other clinical problems which deserve considerable attention. Two of these warrant special mention. First, application of recent knowledge of choline to the treatment of human liver disease is a subject of absorbing interest and unusual promise. Indeed, the concept of cirrhosis of the liver as a deficiency disease is finding growing experimental support from many different directions. Second, calcium absorption, metabolism and excretion have been further clarified. The studies cited in this brief resume are simply representative of the high order of clinical investigation being carried out at the present time. They are indicative of the growing contributions which may be anticipated from those students of the science of nutrition whose efforts are directed toward experimentation on man himself.

In viewing clinical nutrition from the broadest standpoint, it is apparent that the most acute need at present for the assessment of the incidence of deficiency disease is the availability of dependable, relatively simple, objective diagnostic techniques. Since a major part of our national malnutrition is latent, or attended only by vague indefinite symptoms, its certain recognition must depend upon accurate methods of study.

REFERENCES

(1) Kruse, H. D. U. S. Pub. Health Rept. **56:** 1301, 1941. (2) Kruse, H. D. Personal communication. (3) Brenner, S. and L. J. Roberts. Proc. Am. Inst. Nutrition, p. 188, 1942. (4) Ralli, E. P., E. Bauman and L. B. Roberts. J. Clin. Investigation **20:** 709, 1941. (5) Ralli, E. P., E. Papper, K. Paley and E. Bauman. Arch. Int. Med. **68:** 102, 1941. (6) Abels, J. C., A. T. Gorham, G. T. Pack and C. P. Rhoads. J. Clin. Investigation **20:** 749, 1941. (7) Williams, R. D. and H. L. Mason. Proc. Staff Meet. Mayo Clin. **16:** 433, 1941; J. Clin. Investigation **21:** 247, 1942. (8) Robinson, W. D., D. Melnick and H. Field. J. Clin. Investigation **19:** 399, 1940. (9) Robinson, W. D., D. Melnick and H. Field. J. Clin. Investigation **19:** 483, 1940. (10) Elsom, K. O., F. Lukens, E. H. Montgomery and L. Jonas. J. Clin. Investigation **19:** 153, 1941. (11) Shils, M. E., H. G. Day and E. V. McCollum. Am. J. Med. Sc. **201:** 561, 1941. (12) Banerji, G. G. and L. J. Harris. Biochem. J. **33:** 1346, 1939. (13) Jolliffe, N., R. Goodhart, J. Gennis and J. K. Cline. Am. J. Med. Sc. **198:** 198, 1939. (14) Robinson, G. W., P. Shelton and F. V. Smith. Arch. Int. Med. **68:** 945, 1941. (15) Bueding, E., M. H. Stein and H. Wortis. J. Biol. Chem. **140:** 697, 1941. (16) Williams, R. D. and H. L. Mason. Proc. Am. Inst. of Nutrition, p. 192, 1942. (17) Smith, S. G. and D. W. Martin. Proc. Soc. Exper. Biol. and Med. **43:** 660, 1940. (18) Machella, T. E. Am. J. Med. Sc. **203:** 114, 1942. (19) Sebrell, W. H. Vitamin symposium. Univ. of Chicago Press, 1942. (29) Crandon, J. H., C. C. Lund and D. B. Dill. New England J. Med. **223:** 353, 1940. (30) Bryan, A. H., D. F. Turner, R. L. Huenemann and G. Lotwin. Am. J. Med. Sc. **203:** 1942. (31) Heineman, M. J. Clin. Investigation **20:** 467, 1941. (32) Butler, A. M. and M. Cushman. J. Clin. Investigation **19:** 459, 1940. (33) Feller, A. E., L. B. Roberts, E. P. Ralli and T. Francis. J. Clin. Investigation **21:** 121, 1942. (34) Ralli, E. P., G. J. Friedman and S. Sherry. J. Clin. Investigation **18:** 705, 1939. (35) Storvick, C. A. and H. M. Hauck. J. Nutrition **23:** 111, 1942. (36) Heath, C. W. A symposium on the blood. Univ. of Wisconsin Press, 1939. (37) Leverton, R. M. and A. G. Marsh. J. Nutrition **23:** 229, 1942. (38) McCance, R. A. and E. M. Widdowson. J. Physiol. **94:** 148, 1938. (39) Moore, C. V., W. R. Arrowsmith, J. Welch and V. Minnich. J. Clin. Investigation **18:** 553, 1939. (40) Fowler, W. M. and A. P. Barer. Am. J. Med. Sc. **201:** 642, 1941.

(20) Kohn, H. I. Biochem. J. **32:** 2075, 1938. (21) Axelrod, A. E., E. S. Gordon and C. A. Elvehjem. Am. J. Med. Sc. **199:** 697, 1940. (22) Sarett, H. P., J. W. Huff and W. A. Perlzweig. J. Nutrition **23:** 23, 1942. (23) Patton, E. W., W. R. Sutton and J. B. Youmans. J. Clin. Investigation **19:** 785, 1940. (24) Field, H., D. Melnick, W. D. Robinson and C. F. Wilkinson. J. Clin. Investigation **20:** 379, 1941. (25) Watson, C. J. Proc. Soc. Exper. Biol. and Med. **38:** 557, 1939. (26), (27) Kark, R. and A. P. Meiklejohn. New England J. Med. **221:** 519, 1939; Am. J. Med. Sc. **201:** 380, 1941. (28) Beckh, W., P. Ellinger and T. D. Spies. Quart. J. Med. **6:** 305, 1937.

GUIDING PRINCIPLES FOR THE FORTIFICATION OF FOODS

FRANKLIN C. BING

Department of Physiology and Pharmacology, Northwestern University Medical School, and the Office of the Council on Foods and Nutrition, American Medical Association, Chicago

The subject which has been assigned to me for discussion in this evening's symposium can be expressed perhaps best as the Chairman has done when he asked me to take part in the program. He said "Assuming that fortification of foods with vitamin A, B_1, riboflavin, nicotinic acid, and vitamin D is desirable, how should such a program be carried out?" It is well that the Chairman has said that it could be assumed the fortification of foods with certain vitamins is desirable, because that is a debatable subject. Most of us are convinced, however, that the improvement of foods by addition of vitamins or minerals is in the interests of public health, if properly done.

Much has been written about the fortification of foods since the last symposium on the subject sponsored by our Institute of Nutrition (Morgan and others, 1939; Wilder, 1941) just three years ago. Many things have happened, especially the improvement of white flour and bread (Taylor, 1941; Wilder and Sebrell, 1941; Bennett and Itter, 1942) but our views have not yet been clarified. It has been the experience of others and of the Council on Foods and Nutrition of the American Medical Association, which has interested itself in the problems of fortified foods since its beginning in 1931, that many of our previously accepted views have undergone a change. The fortification of foods is an experiment in applied human nutrition, and right now we are in the position of observers of an interesting feeding test which is still in progress.

Regardless of how one views the fortification of foods as compared with the administration of tablets or capsules, certain it is that foods provide an automatic method of securing medication. Despite the knowledge about the antirachitic value of vitamin D, babies occasionally develop rickets because their mothers neglect to continue to give them cod liver oil regularly. Doubtless rickets would be rarer than it is if all milk were fortified with vitamin D. Of course, there will be those persons who will continue to develop rickets despite all ordinary preventive measures, just as there will be those who develop enlarged thyroids despite the availability of iodized salt. For those persons with higher requirements, medical care is of course necessary, but for ordinary persons a fortified food may be considered simply as another item which renders the selection of an adequate diet a little bit easier.

In considering the principles that should serve as a guide in the fortification of foods, it would be well to consider the foods that already have been fortified and the reasons for so doing. Foods that have been fortified with vitamins or minerals or both include iodized salt, vitamin D milk, vitamin A fortified oleomargarine, enriched flour and enriched bread, and some of the special products, such as foods for feeding infants which have been fortified with one or more substances.

Iodized salt. Iodized salt, according to the historical studies of McCay, was suggested as long ago as 1831 by Boussingault. He observed the difference in iodine content of sea salt and the salt from a mine in South America, and the freedom from goiter in one locality and its prevalence in the other, and suggested to the government of Colombia, South America that iodized salt be made available for the prevention of goiter. During the middle of the 19th Century iodized salt is said by McClendon to have been introduced in France, Austria, and Italy but this practice was soon discontinued. For all practical purposes, iodized salt was introduced on a small scale in Switzerland in 1919, shortly after the reports of the value of iodides in the prophylaxis of thyroid enlargement of school children. In this country it was introduced in 1924 in the State of Michigan, as a result of agitation by a committee of the Michigan State Medical Society. This committee secured the cooperation of salt manufacturers and guided them in their efforts to produce a suitable product until 1940, in which year the American Public Health Association was asked to establish a committee to serve in an advisory capacity on a national scale. In 1941 this committee, known as the "Study Committee on Endemic Goiter," was organized.

The amount of iodine selected for iodized salt in this country first was one part of either sodium or potassium iodide to each 5,000 parts of salt. In their studies on the prevention of endemic goiter, Marine and Kimball had shown that thyroid enlargement could be prevented if a school child received a total dose of 400 mgm. of iodine in a year. Assuming that the amount of table salt consumed was about 7.0 grams daily, the amount of iodide in salt which would be necessary to provide 400 milligrams of iodine in a year would be 0.02 per cent. Accordingly, this level was chosen for the United States, although even at the beginning there were some persons who believed that smaller amounts would be equally satisfactory.

Since iodized salt was introduced, some data

have become available on the iodine requirements of man, and it is now considered that the human being needs only about 0.1 mgm. of iodine daily. On this basis two-thirds of a gram of iodized salt containing 0.02 per cent iodide would supply the minimum daily human requirements. The average daily consumption of table salt appears to be from 5 to 8 grams for each person, and about 60 per cent of the table salt sold is iodized. This would indicate that the iodine content of American iodized salt is higher than is necessary.

Recently several outstanding investigators have suggested that it might be well to reduce the amount of iodine in iodized salt to 1 part in 10,000, or 0.01 per cent iodide. The Council on Foods and Nutrition has voted to accept iodized salt containing 0.01 per cent potassium iodide, or an equivalent amount of sodium iodide, provided *a*, the salt uniformly contains this amount when manufactured, and *b*, the salt contains this amount of iodine when standing under ordinary conditions of storage. The former provision can be met by proper mixing, the latter by the use of some of the newer developed methods of stabilizing the iodine content of this product. The Food and Nutrition Board of the National Research Council has approved of iodized salt containing 0.01 per cent iodide.

In summary, therefore, it may be stated that iodized salt was introduced in order to meet a definite deficiency. Other methods have been tried, such as the addition of iodine to drinking water and the administration of iodides to school children but neither of these methods has received quite the general acceptance as has the use of iodized salt. Simple goiter has largely disappeared, so much so that school children today find the word "goiter" an unfamiliar term. Thyroid enlargement occurs but infrequently among our live stock and our domestic pets as as well, and all of this can be attributed to the general consumption of iodized salt. The incidence of exophthalmic goiter likewise is said to have been reduced, but exact statistics are not available and there may be other contributing factors to any reduction of the incidence of this disease. No harm has resulted from the use of iodized salt and, when its consumption has been decreased in a community within the region where endemic goiter has prevailed, the incidence of thyroid enlargement promptly has increased.

Vitamin D milk. As in the case of iodine, there are few foods that are significant sources of vitamin D. Deficiency of vitamin D results in rickets, a condition which ordinarily is observed in infants between the ages of six months and two years. Signs of vitamin D deficiency may appear in older children, in adolescents, and in lactating women. There is much evidence that vitamin D is of little value unless calcium and phosphorus also are ingested in suitable quantities. It is therefore particularly appropriate that milk, which is the richest food source of calcium and is an excellent source of phosphorus, and which makes up a considerable portion of the food intake of infants and small children, should be selected as a food to convey vitamin D. From the practical viewpoint, vitamin D is readily administered when mixed with milk. There is some experimental evidence that the administration of vitamin D dispersed in milk may have other advantages as well.

Vitamin D milk was introduced commerically in 1931 by the Vitex Laboratories which had available a preparation obtained from cod liver oil, and by the Wisconsin Alumni Research Foundation which made available an irradiation method. At first irradiated milk contained only 135 U.S.P. units of vitamin D to the quart, this being approximately as much as could be produced in the milk without developing off-flavors. Improved methods of irradiation since have been developed, however, and irradiated milk containing at least 400 U.S.P. units of vitamin D to the quart is available. The evidence shows clearly that irradiation under actual conditions does not affect the nutritive value of the other dietary essentials in milk. Vitex vitamin D milk from the beginning was made to contain at least 400 U.S.P. units of vitamin D to the quart. Originally it was believed that such milk contained as much vitamin D as three teaspoonfuls of cod liver oil but it was later shown that it is equivalent to about one and one-third teaspoonfuls of cod liver oil, U.S.P. minimum standard. There is abundant experimental evidence to show the value of both irradiated milk and cod liver oil concentrate milk (Jeans, 1936) as well as other forms of vitamin D milk which subsequently have been commercially produced. The claims that can be recognized for these products have been summarized by the Council on Foods and Nutrition of the American Medical Association (1937), which has been actively interested in the scientific aspects of vitamin D milk from its origin. Vitamin D milk containing at least 135 U.S.P. units of vitamin D to the quart usually prevents rickets, and milk containing 400 U.S.P. units of vitamin D to the quart when consumed in the customary quantities used in the feeding of infants, will prevent rickets and promote good growth. Vitamin D milk has other properties that can be attributed to foods supplying vitamin D, as well as the other dietary ingredients of milk.

At the present time it is estimated that only about 5 per cent of the fluid milk supply is vitamin D milk. On the other hand, about 60 per cent of the evaporated milk sold in the country has been

irradiated to yield a potency of at least 135 U.S.P. units to the quart, after dilution with an equal volume of water. In some communities fluid vitamin D milk cannot be sold because local authorites will not permit it, on the basis of conflict with older regulations stating that nothing can be added to milk. In some communities also, vitamin D is considered by the trade as a premium product, which prevents its sale except at premium prices.

The Council on Foods and Nutrition has voted not to accept any milk containing more than 400 U.S.P. units of vitamin D to the quart, because the evidence for the better nutritive value of these higher potencies is not considered conclusive. The Council also holds the view that of all common foods, only milk is acceptable when fortified with vitamin D. Vitamin D fortified cereals are not accepted, although the Council is willing to give consideration to evidence about the value of cereal foods which have been fortified with calcium as well as vitamin D.

Rickets has become so rare that now it is a problem to obtain cases to illustrate the disease to medical students in many of our teaching hospitals. Of course, vitamin D milk cannot assume much of the credit for this lowering of the incidence of rickets, but certainly its ready availability has helped, especially the availability of evaporated vitamin D milk. A number of studies also have been made of the value of vitamin D milk as a source of this dietary essential for older children, adolescents, and for adults.

Thus it is seen in retrospect that the decisions on vitamin D milk were arrived at by a knowledge *a*, that a dietary deficiency due to lack of vitamin D existed and needed to be corrected; *b*, that milk was unique as a vehicle for administration of vitamin D; and *c*, that the amount provided should be such that under ordinary conditions of use it would be difficult to avoid getting enough of the vitamin to prevent symptoms of deficiency.

Oleomargarine fortified with Vitamin A. Oleomargarine fortified with vitamin A now has been on the American market since the Fall of 1937, but before that it had been manufactured in several European countries, in some of which it is compulsory to fortify all oleomargarine with vitamins A and D. The aim in this country was to make the product nutritionally better than the unfortified oleomargarine for the benefit of those persons who buy oleomargarine rather than butter for domestic use. An appreciable proportion of the oleomargarine produced in this country is used in other industries, particularly the baking industry, but this is not considered in the present discussion because all of that product is unfortified. At first a level of 7,500 U.S.P. units of vitamin A to the pound was selected because on the basis of evidence then available, the Council believed that this value represented a fair average of the vitamin A content of butter. During hearings held by the Food and Drug Administration in connection with the formulation of a standard for oleomargarine, evidence was introduced which led to the conclusion that 9,000 U.S.P. units of vitamin A to the pound was a better value for average butter. This was in 1941. Accordingly, products which are produced at the present time have that potency, and some of the manufacturers add an appreciable excess as a factor of safety. Evidence which has been made available to me indicates clearly that the vitamin A potency of fortified oleomargarine remains stable over periods of sixty days and probably longer, under ordinary conditions of transportation and refrigeration.

The Council has made no pronouncement about vitamin D in these products because butter is not a really important source of vitamin D and there seems to be no point in making oleomargarine a better source of vitamin D than is butter. For each 60 units of vitamin A in butter, there is approximately 1 unit of vitamin D. One firm has selected a fish liver oil that contains approximately a 60 to 1 ratio of vitamin A to vitamin D. Other firms have used fish liver oil preparations that have a ratio of 4 to 1, so that the products may contain appreciable quantities of vitamin D. The only claim that the Council recognizes, however, is that such products "contain vitamin D." In other words, the Council's policy is to take no objection to the presence of vitamin D in the preparations used for the fortification of oleomargarine with vitamin A, but to recognize no special claims for this vitamin D.

The merit of such products can be estimated in the following way: The average per capita consumption of butter is about 17 pounds and of oleomargarine a little more than 2 pounds. Suppose a family having to use oleomargarine in place of butter is wise enough to select the fortified oleomargarine, and that they consume about 17 pounds in each year for each person. This would be approximately three-fourths of an ounce daily, or 400 units of vitamin A. This is 10 per cent of the minimum adult vitamin A requirement, an appreciable amount. About half or more of the oleomargarine sold for table use in this country now is fortified with vitamin A and, assuming that supplies of vitamin A are available, it is expected that this proportion will be increased.

Enriched flour and enriched bread. Enriched flour and bread are comparatively new products and have been produced commercially only since the early part of 1941. Already about half of the family flour sold in the United States and about one-third of the baker's bread are enriched. The

ideas behind the fortification of flour and bread have been described well by a number of writers, but I like to consider the subject from the following point of view: There is evidence of deficiency of thiamine, riboflavin, nicotinic acid, calcium, and iron in the diets of many American people. There are foods which will serve as excellent sources of some of these factors. Thus milk is an excellent source of riboflavin and calcium. Meats, especially pork, are excellent sources of thiamine, riboflavin, and nicotinic acid. Both meats and whole grain cereals provide appreciable quantities of iron and thiamine. Unfortunately these foods either are considered expensive or, as in the case of whole grain cereals, the public has not accepted them wholeheartedly. It would seem to be desirable to add thiamine, riboflavin, nicotinic acid, iron, and calcium to a food product which is cheap and which is regularly consumed by most of the people whose diets are apt to be deficient with respect to these factors. What can be a better vehicle for this purpose than white flour and white bread? These are wholesome foods even without

TABLE 1

*Important food values of natural grains and upper limits of "restoration" for general purpose cereal foods**
(Council on Foods and Nutrition, American Medical Association)

PRODUCT	Ca	Fe	THIAMINE	RIBOFLAVIN	NIACIN
	Mgm. per 100 Calories				
"Restored" Cereal.....	75	1.5	0.25	0.10	
Cornmeal.....	7.2	0.9	0.065	0.05	0.4
Oatmeal.....	26	1.2	0.24	0.05	0.4
Whole Wheat Flour.....	14.8	1.4	0.13	0.07	1.8

being improved, but their industrial development since about 1870 is considered by many persons to have contributed to the defects of our national diet. Enriched flour and enriched bread are preferred foods. They help make it easier for a person to select a diet adequate in a number of essentials. Whether it will help solve the problem of the more severe thiamine and nicotinic acid deficiencies remains to be seen.

Enriched flour according to the standards enunciated by the Food and Drug Administration must contain in each pound not less than 1.66 mgm. nor more than 2.5 mgm. of thiamine, not less than 1.2 mgm. nor more than 1.8 mgm. of riboflavin, not less than 6 nor more than 9 mgm. of nicotinic acid or its amide, and not less than 6 mgm. or more than 24 mgm. of iron. Optional ingredients are vitamin D and calcium. The complete provisions are provided in the announcement published in the Federal Register for April 1, 1941.

It is evident that the standards represent a compromise of several conflicting views presented at public hearings conducted by the Food and Drug Administration. Enriched flour is nutritionally superior to white flour but the minimum standard is below the values of whole wheat flour. However, even enriched flour of minimum standard composition has valuable potentialities as is

FOOD
QUANTITY
Milligrams of Thiamine (Average Values)
Whole Wheat Bread 6-OZ. 0.51
White Bread made with Enriched Yeast * 6-OZ. 0.38
White Bread made with Enriched Flour * 6-OZ. 0.38
White Bread made with White Patent Flour 6-OZ. 0.056
Milk 1-PT. 0.26
Meat 4-OZ. 0.50
Egg 1 0.07
Potato 4-OZ. 0.15
Citrus Fruit 4-OZ. 0.11
Tomato 4-OZ. 0.12
Other Vegetables 4-OZ. 0.20
Other Fruits 4-OZ. 0.056
Breakfast Cereals (Whole Grain) 1-OZ. 0.10

Chart 1. Thiamine content of bread and other foods commonly included in an adequate diet. The chart shows that whole wheat bread or enriched bread, when substituted for white bread, is capable of making significant contributions of thiamine to the diet.

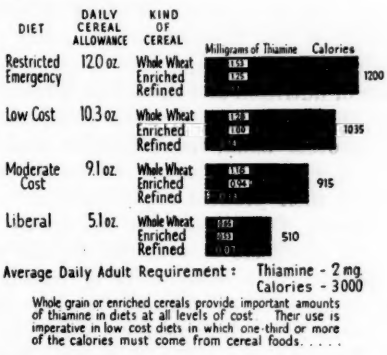

DIET
DAILY CEREAL ALLOWANCE
KIND OF CEREAL
Milligrams of Thiamine
Calories
Restricted Emergency 12.0 oz. Whole Wheat Enriched Refined 1200
Low Cost 10.3 oz. Whole Wheat Enriched Refined 1035
Moderate Cost 9.1 oz. Whole Wheat Enriched Refined 915
Liberal 5.1 oz. Whole Wheat Enriched Refined 510
Average Daily Adult Requirement: Thiamine - 2 mg. Calories - 3000
Whole grain or enriched cereals provide important amounts of thiamine in diets at all levels of cost. Their use is imperative in low cost diets in which one-third or more of the calories must come from cereal foods.

Chart 2. Thiamine from cereals. The chart shows the significant contribution to the thiamine content of the diet, particularly of economical diets, possible if the cereal foods have the nutritive value of enriched or whole wheat flour, as compared to the insignificant thiamine contributions of cereals having the value of white flour. The diets are those described in "Diets to fit the family income," R. S. Carpenter and H. K. Stiebeling, Farmers Bulletin, 1757, U. S. Department of Agriculture.

indicated in the accompanying charts 1 and 2. A discussion of the subject has been provided by the Council on Foods and Nutrition (1941).

General problems arising from the fortification of foods. As a result of experience to date, we know that a number of unsettled problems have arisen

about the fortification of foods. There are three groups concerned, the public, the manufacturers or other representatives of the food industry, and nutritionists and medical scientists and others interested in the scientific aspects of public health. Scientists and manufacturers share responsibilities to the public.

Fortunately, the questions involving the general public can be readily answered. Intelligent consumers want to know whether vitamins and minerals actually are in the fortified foods, they want information regarding the usefulness of the food, and the like. Foods which have been improved by the addition of vitamins or minerals, or both, should be standardized, they should be informatively labeled and truthfully advertised. There are, of course, provisions which afford reasonable assurance that these things are taken care of.

On the part of the manufacturer, the question sometimes is raised why scientific groups should approve of the fortification of one food and disapprove of the fortification of another. It can readily be understood that the fortification of foods gives tremendous advertising value to the products. It is worth a great deal to a manufacturer to have nutritionists or physicians go on record that the type of food which he is selling is the kind of food that one ought to buy. The milk industry long has enjoyed boosts from serious students of nutrition and the cereal industry now is receiving a fair share of scientific approbation.

On the other hand, manufacturers sometimes ask why they should be asked to bear the burden of making their product an instrument for the improvement of the health of the public. Not all of the producers of evaporated milk fortify their product with vitamin D or irradiate it. Indeed, some of them attempt to make capital out of the fact that their products are not so fortifield. Not all of the millers have enriched their flour, especially the millers of cheaper grades of flour used by consumers who need a more nutritious product. It would seem that there is a problem not only of answering the question why certain particular products are selected, and ought to be fortified, but also of stating why certain products are not approved for fortification purposes.

Let us not forget the broader problem of public health in discussing the details of the merits and demerits of any fortification program. As nutritionists we may have to be more or less arbitrary in making specific recommendations, or at least in making selections about foods that should be and should not be chosen as vehicles for vitamin and mineral enrichment, but we can be consistent with regard to our desire to improve the public health.

The food manufacturer who sincerely desires to improve his product by addition of vitamins or minerals, and whose product has not been selected by unbiased groups of nutritionists as a desirable food for fortification should first make a thorough investigation of the nutritive value of his product at different stages of processing or manufacturing. It is important for a food manufacturer to observe *a*, that his product be safe to eat, free from pathogenic or spoilage organisms, and not contaminated with foreign materials; b, that the nutritive value of the product be retained to as high a degree as possible, consistent with necessary processing for purposes of preservation and safety. Then if a food product is found unavoidably to have become nutritionally damaged during processing it becomes eligible for improvement by addition of vitamins or minerals. If the particular item of food is one that is relied on as an important source of a dietary essential, if consumers are led to believe that they can obtain some dietary essential in significant amounts from that food, then it should be appropriate for the manufacturer to put back what has been lost if this can be done adequately and economically. That essentially is the restoration idea, which has been much criticized, but which has so many advantages from the educational point of view that it should not be wholly abandoned. Any advertising claims should be restricted to simple statements of fact such as that the restored food product has the full nutritive value of the unprocessed food with respect to the substances added.

As a guide to manufacturers of breakfast cereal foods the Council on Foods and Nutrition has adopted the following decision: Restoration with thiamine, riboflavin, and iron salts is permitted, for an accepted product, up to the highest values found in common cereal foods. Mild fortification with calcium salts is also permissible, for the simple reason that no cereal food is a significant source of this element and, if added, it should be to an amount that conveys some nutritional merit to the products. Table 1 shows the values for a number of cereals and for an acceptable breakfast cereal, in terms of milligrams per 100 calories, which is about the same as milligrams per ounce or per serving. A number of commercial products have been enriched along these lines.*

* Because of current interest in nutrition the question of nutritional restoration of processed breakfast cereal foods again has been raised. No doubt many practical problems will need solution before final decisions are made. I believe that it would be in the interests of the public to permit manufacturers of breakfast cereal foods to restore their products so that each ounce of the finished

Should a manufacturer wish to go beyond natural levels of fortification, then it is particularly necessary for him to have unbiased medical and scientific opinion to justify the procedure he desires to follow. Presumably, the product might be a special purpose food, a food designed for some particular purpose, such as a cereal to be used in the feeding of infants or a food adjunct to be consumed in restricted diets. Then it would be quite appropriate for such special dietary foods to have incorporated in them one or more of the dietary essentials, to the extent that a daily portion of the product would provide up to the full daily requirements of the particular group for whom the product is designed. It is necessary, of course, that the product provide the vitamins and minerals which are claimed for it under ordinary conditions of storage and use. The use for which the item is intended should be clearly emphasized in the labeling and advertising.

The number of restored foods and of foods intended for special dietary use may be large, because the impetus for the production of these products presumably comes from the manufacturers. But for fortified foods, that is, staple items which have been manipulated for the purpose of fitting into a program for the improvement of the public health, there can be but a limited number of products, because these ought to be sponsored by scientific groups, in cooperation with manufacturers. They should not be purely commercial undertakings.

In this connection it may be of interest to quote from the "findings of fact" made from the record of the hearings of the Food and Drug Administration on enriched flour:

"In general, indiscriminate enrichment of food (including flour and self-rising flour) with vitamins and minerals would tend to confuse and mislead consumers by giving rise to conflicting claims regarding the beneficial effects of such substances, and would be likely to lead to the impression on the part of consumers that a single article of food so enriched would meet all nutritional needs.

"Limited enrichment of selected staple foods, on the other hand, is readily adapted to the promotion of consumer understanding of the relative value of enriched and natural foods, utilizing such education as consumers have received regarding the nutritive value of natural foods." (From Federal Register, Tuesday, April 1, 1941.)

It seems to me that the following conditions should be fulfilled before fortification of a staple food is recommended: *a*, there should be a reasonable amount of evidence to show that a deficiency of the particular vitamin or mineral or other dietary essential exists, or that for different groups it is difficult to secure an adequate intake of the factors in question; *b*, it should be shown that the food product in question is a suitable vehicle for the vitamin or mineral or other dietary essential. The added material must be stable under ordinary conditions of storage and use. *c* The food product which is to serve as a vehicle should be one that frequently is used; that is readily available, particularly to those in low income groups. In every case, the intent should be to select the food so as to make it as difficult as possible for the general public to fail to get a sufficient quantity of the dietary essential which is added to the food; *d*, no restrictions should be placed on the food selected because that particular food ordinarily does not contain the dietary essential in question. In fortifying foods we are doing something that is a little bit unnatural, and therefore it is not essential to select natural vehicles. On the other hand, the natural vehicles usually are those which can retain their vitamins well during storage; *e*, the amounts of the vitamins, minerals, or other dietary essentials added should be sufficient to make it reasonably certain that the dietary deficiencies which led to the formulation of the fortified food will be corrected. Evidence should be sought to determine the value of the fortified product.

The details of our thinking about the fortification of bread or flour and other foods appear to be essentially unimportant. The real problem remains to be answered; that is whether the products that have been developed will really do what we believe they can do, help improve the nutritional status of the people. To my mind, it may be necessary in some of the Southern communities where pellagra is endemic, to have available an additional special purpose food which is rich in nicotinic acid, and in other members of the vita-

---

product will provide 1.5 mgm. of Fe, 0.25 mgm. of thiamine, and 1.8 mgm. of niacin. Riboflavin might well be left for further consideration after supplies of this substance become available. The addition of calcium salts might well await the results of experiments to determine the amounts that can be added without affecting the taste of the products. In my opinion all "restored breakfast cereals" should provide the same amounts of iron, thiamine, and niacin. This will avoid conflicting claims of producers and conflicting impressions of consumers about the relative merits of products made from corn, oats, wheat, rice, etc. The manufacturer of oatmeal will not have to add any thiamine; the manufacturer of a whole wheat product that is nutritionally unimpaired will not have to add any nicotinic acid. The manufacturer of breakfast cereal made from corn may have to add a number of things to bring his product up to the required level for a "restored breakfast cereal food."

min B complex. There is no reason also why manufacturers of enriched flour or enriched bread could not add more of the various dietary ingredients than the minimum standards provide for. I should think it would be good business for them to add amounts in sufficient quantities so that they can say in their advertising "Our enriched flour contains as much thiamine, riboflavin, nicotinic acid, and iron as you can expect to find in the best whole wheat flour." As already stated, the enriched flour now on the market is minimum standard and such a product really is a partially restored food rather than a fortified product. It must be considered as a fortified product however, because its definition permits of improvement far beyond the natural levels of whole wheat.

Enriched flour, minimum standard, contains about 80 per cent of the thiamine content of hard wheat, about one third of the iron content, and about a fifth of the nicotinic acid content of the whole grain. When riboflavin is available in sufficient quantities enriched flour will have about the same content of this dietary essential as whole wheat. Standards for enriched bread have not yet been promulgated, but it is assumed that in general it will be possible to make enriched bread by using enriched flour.

The Chairman has asked questions specifically about the fortification of foods with vitamin A and vitamin C. There is evidence that an appreciable number of persons show signs of a deficiency of vitamin A, and there is evidence that for an appreciable number of persons their vitamin C intake might well be increased. I think the answers to these questions are about as follows: In the case of vitamin A, we have many valuable natural sources. Vegetables, especially the green leafy and yellow vegetables, both fresh and canned, are excellent sources of this factor. Oleomargarine fortified with vitamin A helps solve the decrease in vitamin A of the diet of those persons who substitute that product for butter. It should be easy to correct any deficiency of vitamin A by encouraging the consumption of naturally rich sources which are everywhere available. Further, these particular foods offer other valuable nutrients as well as carotene.

With regard to vitamin C, I think the answer is about the same. We have so many naturally rich sources of this factor that we do not need to look for artificial preparations, except for medical purposes or for the treatment of persons who are allergic to certain fruits that are the ordinary sources of vitamin C. In addition to citrus fruits and tomatoes, we have many humble foods such as cabbage and turnips, which are outstanding sources of this vitamin.

With regard to the other dietary essentials, it is seen that something has been done for iodine deficiency, and endemic goiter has almost ceased to be an important problem of public health. Something has been done to overcome the natural deficiency of most foods in vitamin D. I think it can be concluded that vitamin D milk, with other sources of vitamin D, gives us as many agents for the prevention of vitamin D deficiency as are necessary. Enriched flour and enriched bread help take care of thiamine, riboflavin, and nicotinic acid deficiency, and it still remains to be seen how effective this step will have been. Should it prove not to be effective, then the problem will need further investigation and a higher level of enrichment requested, or some other method of treating the deficiency on a large scale will have to be attempted.

These represent my personal views on these subjects at the present time, and they do not necessarily reflect the opinion of the Council on Foods and Nutrition or of the Food and Nutrition Board. I trust that this narration has not added to the confusion of thought about a difficult problem in applied nutrition.

REFERENCES

BENNETT, H. C. AND S. ITTER. Hygeia **20:** 197, 1942. Council on Foods and Nutrition of the American Medical Association. J. A. M. A. **116:** 2849, 1941. Council on Foods and Nutrition of the American Medical Association. J. A. M. A. **108:** 206, 1937. JEANS, P. C. J. A. M. A. **106:** 2066, 2069, 2150, 1936. MORGAN, A. F., L. J. ROBERTS, W. H. SEBRELL, E. M. NELSON AND A. E. TAYLOR. The Milbank Memorial Fund Quart. **17:** 221, 1939. TAYLOR, A. E. Wheat Studies of the Food Research Inst. **18: 77**, 1941. WILDER, R. M. AND W. H. SEBRELL. Nutrition—the armor of robust health. Published by The Nutrition Advisory Committee to the Coördinator of Health, Welfare, and Related Defense Activities, Washington, 1941. WILDER, R. M. Ann. Int. Med. **14:** 2189, 1941.

THE LABORATORY CONTROL OF ENRICHED FOODS

BERNARD L. OSER

Food Research Laboratories, Inc.

Advances in methodology during the past decade have been responsible in no small measure, not only for the discovery, isolation, and ultimate synthesis of vitamins, but also for the introduction in food and pharmaceutical products of those vitamins whose relationship to human nutrition has been more or less well established. Time was when the only available methods for the appraisal of vitamin content were those involving biological assay. Delays and expense inherent in such assays naturally impeded progress in the practical application of the so-called newer knowledge of nutrition.

In order to control fortified foods with respect to compliance with Federal and State laws, it has become necessary to establish standards of enrichment as well as official and plant control methods of assay. Official and tentative methods of assay have been and continue to be elaborated through the sponsorship of such groups as the U. S. Pharmacopoeial Revision Committee, the Association of Official Agricultural Chemists, and such organizations as the Research Corporation, the Animal Vitamin Research Council, and the American Association of Cereal Chemists.

To illustrate how the supremacy of Mus norvegicus has been challenged by the connivance of the chemist and the bacteriologist, the following table is presented.

This is by no means a complete tabulation of the many methods that have been proposed for the assay of vitamins, but is submitted merely to illustrate the point insofar as it relates to those vitamins that have achieved prominence in connection with the fortification of foods. Only methods which have actually been used successfully in practice have been included. Numerous minor variations of these procedures have been proposed. In some cases, like the spectrophotometric method for vitamin D, procedures have been reported which are applicable to certain specific natural or synthetic materials but they have not been developed to the stage where they can be used generally in the food control laboratory.

For the purpose of this classification the fluoro-

TABLE 1

Practical vitamin assay procedures

VITAMIN	BIOLOGICAL	CHEMICAL	PHYSICAL	MICROBIOLOGICAL
A	Rat growth (*USP*)	Carr-Price Reaction ($SbCl_3$)	Spectrophotometric ($E^{1\%}_{1cm}$. 328 millimicrons	
B_1	Rat curative (*USP*) Rat growth (AOAC collaborative) Pigeon curative	Thiochrome Reaction (Fluorometric) Azo Dye Reaction (Colorimetric)		Fermentation (yeast) Mycologic (P. blakesleeanus)
B_2	Rat growth (Mod. Sherman-Bourquin)		Fluorometric	L. helveticus (Snell-Strong) (Acid formation) (Turbidimetric)
C	Guinea pig growth Guinea pig curative (symptom score) Guinea pig tooth histology	Indophenol Dye (Titrimetric) (Colorimetric) Iodine (Titrimetric)		
D	Rat curative (*USP*) Chick preventive (*AOAC*)	Spectrophotometric (?) ($SbCl_3$)		
Niacin	Dog growth Dog curative (Black-tongue)	CNBr + Amine Reaction (Colorimetric)		L. arabinosus (Snell-Wright) (Acid formation) (Turbidimetric) Paradysentery bacillus (Dorfman-Koser) (Turbidimetric)

metric method for thiamine has been regarded as a chemical method, since the vitamin undergoes a chemical transformation, whereas the fluorometric method for riboflavin is regarded as a physical method.

With this choice before him it is incumbent upon the analyst to exercise considerable discretion in the selection of a method. In practice it is not feasible for each assayer to make a critical appraisal of all available methods for a given vitamin. Hence, in arriving at a selection it is necessary to take into account such factors as the special skill or equipment necessary, the time and cost of carrying out each assay, the accuracy or specificity desired, and the degree of precision warranted by the purpose for which the assay is to be conducted.

Vitamin assays which form the basis of evaluation of raw materials entering into commercial channels often sacrifice the reliability of the biological assay for the speed and closer precision of the more recent methods. It is usually understood and often specified in commercial transactions that vitamin unitage established by one of the short methods, e.g., by the spectrometric method in the case of fish liver oil, shall be subject to confirmation by the official biological assay. The chemical, physical, and microbiological procedures are indispensable to the proper control of a product in its transition from raw material to the final stage. These methods also find considerable application in the manufacturer's control of the stability of his product as determined from tests of samples obtained on the open market.

In order to justify the claims for vitamin unitage expressed or implied on the label of his product, a manufactuer must assume responsibility that the vitamins will be found to be present in the amounts claimed when assayed by official procedures which are, as a rule, biological. It is essential, therefore, that correlation between the biological assay and the laboratory control procedure be established in order to furnish support for such claims. Indeed, frequent biological assays should be made as a matter of good control practice. The consumer is entitled to expect biological activity of a certain kind and degree from the vitamins declared to be present in the foods he buys. It is chiefly for this reason that official assay methods employed in enforcement of the legal statutes are based on biological effects characteristic for each vitamin.

This raises the critical question of the distinction between the total and available vitamin content of a food. Of late years, the literature has contained repeated evidence of the inefficiency with which the animal organism utilizes carotene, the precursor of vitamin A. Is one justified, therefore, in estimating "carotene" by a colorimetric method and computing the result in terms of vitamin A unitage? Similarly, evidence has been adduced to show that vitamin A alcohol is biologically not as effective as the ester. In some instances wide discrepancies have been noted between the vitamin A content estimated spectrometrically and biologically in a product containing the unsaponifiable fraction from fish liver oil. Is it always correct to translate the extinction coefficient directly into biological units? It has been reported, and indeed confirmed at this very meeting, that the thiamine and other water-soluble vitamins in moist compressed yeast is incompletely absorbed and utilized. It is, therefore, proper to ask whether methods which determine the total vitamin content of fresh yeast can be employed to justify claims for potencies of presumably physiologically available vitamins in fresh yeast.

Another aspect of this problem concerns the form in which the product is consumed. For example, efforts to enrich macaroni with water-soluble vitamins have been confronted by a culinary obstacle, namely, the extraction of much of the vitamin by the cooking water. However, the vitamins claimed to be present in uncooked macaroni can be demonstrated by analysis.

An experience amusing to us, if not to the manufacturer, concerned a vitamin D-fortified chewing gum. The expected amount of vitamin D could indeed be demonstrated by biological assay in the pulverized sample. We suggested, however, that it might be worth while investigating whether the vitamin was extracted from the gum by saliva during the chewing process. Assays of the gum wad after 20 minutes of chewing showed that the vitamin had not been extracted. Was the manufacturer morally or legally justified in declaring this product to be a source of vitamin D?

It is well recognized today that the total nitrogen content of foods is not an accurate index of the biological availability of the protein; that the total iron in the ash does not truly represent hematopoietic iron; that so-called "carbohydrates by difference" are not completely utilized as a source of energy. And so it appears, at least in cases like those cited above, that vitamins are no exception to the adage "all that glitters is not gold."

It may be of interest to dwell for a moment on the question of sampling. Raw materials, intermediates, and finished products are usually tested in plant control laboratories as a matter of routine. In addition, many companies examine their products for stability of the vitamin content, not only under exaggerated conditions in the laboratory, but by analyses of field samples at various stages of their shelf life. It is essential, of course, that containers be properly coded to show the date and the plant in which the goods were packed.

It is sometimes possible to determine the temperature coefficient for the deterioration of vitamin content, thus permitting a definite prediction of shelf life from data obtained at elevated temperatures in the laboratory tests. It is usually found, however, that where several vitamins are present the rate of destruction will differ for each vitamin. In such cases the fate of one of them, the most labile, should be adopted as the index of shelf life. Samples are frequently submitted to outside laboratories for independent confirmation of plant control data. Often these laboratories are asked to conduct or observe the sampling. Many manufacturers of vitamin-fortified foods make it a policy to authorize open market purchases of their products at irregular, unannounced intervals for the purpose of providing an independent check of the validity of their vitamin claims. This is provided for by municipal or state laws in the case of vitamin D milk and is a condition generally required by licensors of fortification processes. The granting of seals of acceptance by such agencies as the Council of Foods of the American Medical Association is often contingent upon regular independent check assays.

In the collection of open market samples from retail sources throughout the country the cooperation of the telegraph companies has proved invaluable. Through the commercial service department of Western Union we have been able to have purchases made at specified times wherever there is a telegraph office. Information is furnished by the local manager of the time, place, price, etc. of each purchase. For the collection of whole milk, special shipping containers are forwarded by the laboratory prior to the date of collection and the milk is returned by express in the iced shipper.

In the enforcement of the Federal Food, Drug, and Cosmetic Act and of similar state laws, government agents are authorized to collect samples for examination or analysis. Sections 304 (c) and 702 (b), and the regulations thereunder, contain provisions governing the collection of such samples and specifically authorize the Administrator to "provide a part of such official sample for examination or analysis by any person named on the label of the article, or the owner thereof, or his attorney or agent." Regulations also cover the size of sample which must be collected and its ultimate disposition.

For legal purposes, it is of course essential that every individual package or container be correctly labeled with respect to its contents. It is insufficient for a vitamin claim to be based on the average potency since this would permit deviations below the level claimed. In commercial practice, therefore, it is necessary to provide a reasonably safe margin of excess over the claimed vitamin content, to allow for the analytical or biological error of assay. Let us take the case where the error of an assay is distributed about the mean in accordance with the normal frequency curve. It is obvious that a single assay conducted at the level of the mean should have an even chance of failing even though the claimed content of vitamin may actually be present. In order to determine how much "overage" of vitamin to provide in his product, the manufacturer should take into account the magnitude of the error of the assay. Thus, if the "overage" is equal to one standard deviation then only that fraction of the area under curve B falling to the left of line M represents the chances of failure of a single assay. Theoretically, this would be one chance in 6. If the "overage" were equivalent to two standard deviations, the probability of failure would be equivalent to the ratio that the area

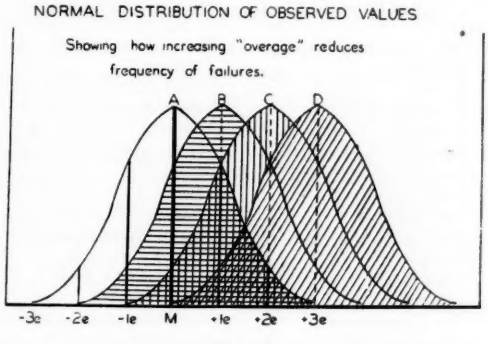


FIG. 1

under curve C lying to the left of line M bears to the total area, viz., one chance in 44. Thus, the "overage" to be allowed may be determined from the error of assay and probability tables on the basis of the chance the manufacturer is willing to take of the failure of an individual check assay, conducted in a government laboratory or elsewhere.

Practices vary with regard to the collection of a fractional sample representing a large number of units or containers. In the case of valuable materials such as high potency fish liver oil it is customary to collect a composite sample representing 100 per cent of the drums. Ordinarily, however, a good rule is to prepare a composite from the square root of the number of containers but never less than ten. This is the procedure recommended, for example, by the Association of Official Agricultural Chemists in sampling sacks of flour.

A wide variety of foods has been considered in relation to laboratory control of vitamin content. Many of these represent foods to which vitamins

have been artificially added in the form of synthetics, concentrates, extracts or potent natural sources. Fortification of foods with vitamins has, in a few instances, reached the level of absurdity while at the opposite extreme are enriched foods which have received official blessing. Table 2 is based on one which appeared in "Food Industries" and shows the wide variety of foods which have been considered from this standpoint. Products for which standards of enrichment have been or are about to be established are indicated in italics. The levels of enrichment officially adopted or under consideration are indicated in table 3.

The standards for vitamin D milk, vitamin A fortified margarine, and enriched flour and farina are now in effect, except that the date of enforcement of the mandatory riboflavin provision for flour and farina has been postponed until

TABLE 2

Foods to be considered from viewpoint of vitamin control

Beer	Fruit juices, canned
Biscuits	Ice cream
Bread	*Infant foods*
Breakfast foods	Lard
Butter	Macaroni
Cakes	Malt tonics
Carbonated beverages	*Margarine*
Carbonated water	*Milk*
Confectionery	Noodles
Cookies	Prepared shortenings
Crackers	Spaghetti
Dried fruits	*Special dietary foods*
Dried milk	Sugars
Dried vegetables	Vegetable juices, canned
Evaporated milk	Vegetable oils
Flour	Wine
Frozen fruits	*Yeast*
Frozen vegetables	

about July 1. Unless the shortage of this material is relieved soon, it is possible that the effective date may be extended further. The Food and Drug Administration has held lengthy hearings on the standards and definitions for bread. While no official standards have been issued to date those tabulated above have received most favorable consideration and are in current use.

Time necessitates limiting this discussion chiefly to the more popular assay procedures as used in our laboratories to control the enriched foods listed in the preceding table.

Margarine is fortified with vitamin A by the addition of fish liver oil or fish liver oil concentrate to the fat or oil of animal or vegetable origin. The finished product contains in each pound 9,000 U.S.P. units of vitamin A plus whatever "overage" the manufacturer allows. The stages in the process at which vitamin control is necessary are indicated in table 4.

Spectrophotometric methods are employed to check the vitamin A content of the fish liver oils or concentrates although most manufacturers require biological confirmation from the supplier. Since the margarine oil, which constitutes not less than 80 per cent of the finished product, is fortified to a level somewhat over 30 units of

TABLE 3

Official standards for enriched foods

FOOD	VITAMINS OR MINERALS	LEVELS
	Mandatory	
Flour (Including bromated and self-rising)	Thiamin	1.66–2.5 mgm. per lb.
	Riboflavin*	1.2–1.8 mgm. per lb.
	Niacin	6–24 mgm. per lb.
	Iron	6–24 mgm. per lb.
and	*Optional*	
	Vitamin D	250–1000 U.S.P. units per lb.
Farina	Calcium	500–2000 mgm. per lb.
	Wheat germ (Farina only)	≯8 per cent
Milk		
Whole	Vitamin D	135 or 400 U.S.P. units per qt.
Evaporated	Vitamin D	7.5 U.S.P. units per av. oz.
Margarine	Vitamin A	≮9,000 U.S.P. units per lb.
	Mandatory	
	Thiamin	1.1–2.2 mgm. per lb.
Bread	Riboflavin	0.6–1.2 mgm. per lb.
(Under consideration)	Niacin	10–20 mgm. per lb.
	Iron	8–16 mgm. per lb.
	Optional	
	Vitamin D	150–600 U.S.P. units per lb.
	Calcium	300–1200 mgm. per lb.

* Enforcement postponed.

TABLE 4

Laboratory control of fortified margarine

Vitamin A Oil:
- (a) Spectrophotometric
- (b) U.S.P. Bioassay

Margarine Oil:
- $SbCl_3$ method
  1. Qualitative (factory) control
  2. Quantitative (laboratory) control
     Adaptation of Dann-Evelyn method*

Margarine:
- (a) $SbCl_3$ method (as above)
- (b) Spectrophotometric method involving ultraviolet destruction of vitamin A.
- (c) U.S.P. Bioassay

* Saponify 2–4 gram sample. Extract with ether. Wash, dry, evaporate under CO_2. Dissolve in $CHCl_3$. Dilute to 10 cc. Test 1 cc. aliquot containing about 5–15 units. Use blank and known increment in evaluating results. Use brown glassware.

vitamin A per gram, the spectrophotometric method is not very satisfactory unless it can be carried out on the unsaponifiable fraction of the margarine oil. Unless this is done the blank due to irrelevant absorption of the fat or oil at the characteristic wave length for vitamin A is too high.

The Carr-Price method has been useful in this connection not only because it affords greater sensitivity than the measurement of the extinction coefficient but because it can also be used as a qualitative plant control method. Factory employees are trained to test the margarine oil to check whether the vitamin A has been added.

The Dann and Evelyn adaptation of the antimony trichloride method has proved adaptable to the quantitative control of margarine. It will be noted that the procedure described in the footnote to table 4 suggests the use of a blank to which no reagent has been added in order to correct for interfering color or turbidity. It is also advisable to determine the photometric density produced by the addition to an aliquot of the chloroform solution of a known increment of vitamin A. The purpose of this is to compensate for any change in the reagent or inhibition of the reaction which may occur in samples of unknown composition. While a truly linear relation between vitamin A increment and photometric density may not always be observed, only a small error results from assuming such a relation to exist within narrow limits. In working with solutions of low vitamin A content, it is essential to avoid undue exposure to light. In this connection brown glassware is useful.

Taking advantage of the fact that ultraviolet light will destroy vitamin A, Neal, Haurand and Luckmann have devised a method for measuring vitamin A content of butter spectrophotometrically. To correct for non-specific interference they measure the residual absorption after irradiation for a long enough period to cause disappearance of the yellow color of carotene and a negative antimony trichloride reaction. This method may also be applied successfully in the estimation of the vitamin A content of fortified margarine.

In the biological assay of margarine, it is important to exercise care in the preservation and preparation of the sample. Because the calculated daily dose of margarine is somewhat less than 100 milligrams it is laborious and time-consuming to weigh the daily supplement directly. Besides animals often do not consume the entire dose within the day. We have found it convenient to prepare fresh each day a pasty dilution of the margarine in a mixture of equal parts of confectioner's sugar and cornstarch which the rats eat readily. One-half gram doses are easily weighed out.

It should also be pointed out that it is not possible to make accurate determinations of vitamin A content from control assays in which both the assay material and the reference oil are fed at single levels. Such determinations of unitage are not required when the assay is conducted to confirm a minimum claim. Further study should be made of the U.S.P. biological assay for vitamin A to determine how it may best be adapted to quantitative estimation of unitage. Attention should be directed to the minimum number of assay and reference groups to be employed, the spacing of the desired growth response, interpretation of non-linear responses, etc. In fact, a thorough statistical study should be made of several official bioassay procedures employing the new methods of analysis of variance to establish limits of precision and the degree to which each variable contributes to the error. It seems possible that such studies can lead to bioassays of greater precision.

Procedures employed in our laboratory for the control of enriched bread are indicated in table 5. The sample should consist of at least three loaves representing the early, middle and late parts of the run. A representative portion is taken from each loaf. If quarter loaves are used, it is essential that the proper relationship of crust to crumb be maintained by cutting the loaf both laterally and longitudinally. The sample is air-dried, care being taken to avoid loss of riboflavin by exposure to light.

Where speed is desired, it is possible to avoid preliminary air-drying of the bread by preparing in a Waring Blendor a uniform acid-aqueous suspension of a representative portion of the loaf. This procedure has the disadvantage that the prepared sample does not keep well. However, another aliquot can be dried concurrently for moisture determination and for preservation as a reference sample.

Distribution of the vitamins and minerals added for enrichment is not as uniform in flour as in bread. For this reason it is desirable to use a large (20 grams) sample of flour suspended in acidulated water and to take aliquots of this suspension for the analysis.

Aliquots of the air-dried bread sample are subjected to analyses for moisture, by drying at 130°, for iron by the A.O.A.C. colorimetric thiocyanate method; for thiamine by the modification of the thiochrome procedure developed in a collaborative study under the auspices of the Research Corporation; for riboflavin by the Andrew's diastase modification of the Snell-Strong microbiological method involving titration of acid produced by L. helveticus; for niacin by the colorimetric method developed in the collaborative study sponsored by the Research Corporation.

Special precautions must be exercised to insure complete extraction of thiamine from dried bread samples. The vitamin is extracted with greater difficulty from bread than from enriched flour. The relation of volume of acid to the size of the sample is also important as will be seen in figure 2. When decreasing quantities of the air-dried sample were subjected to extraction by the same volume of acid, increasing thiamine values were obtained. A one gram sample gave a value 60 per cent greater than a 6 gram sample. Recovery experiments yielded theoretical values irrespective of the size of the sample.

Collaborative riboflavin analyses conducted upon cereal products have shown that results by the microbiological method are usually more reliable than those obtained by the fluorometric procedure. This is due in part to the low riboflavin content of such products. It is essential in the microbiological test that the aqueous extract of cereal products be treated with a diastase since colloidal starch appears to have a stimulating effect upon the microorganism causing greater acid production.

In the case of nicotinic acid assays the tentative collaborative method outlined here yields essentially the same results as the microbiological procedure provided preliminary hydrolysis is employed to convert nicotinic acid precursors to a

TABLE 5

Preparation of enriched bread samples

3 Loaves
(from start, middle and end of run)

½ Loaves
Weigh. Air-dry in dark.

Reweigh (per cent loss). Grind to 40-mesh.

Moisture	*Iron*	*Thiamin*	*Riboflavin*	*Niacin*
2 grams sample	5 grams	2 grams	10 grams	1 gram
2 hr.—130°C.	Ash	Extract with	Extract with	Hydrolyze with
		75 cc. 0.1 N	100 cc. H_2O in	15 cc. N HCl
		H_2SO_4 under	autoclave	
		CO_2. 100°C, 30′.	15 lb., 30′.	Adsorb on
	NH_4CNS			Lloyd's reagent
	Method	Adjust to pH4.7	Diastase	
			50C°, 30′.	Elute (NaOH)
		Phosphatase		
			Microbiological	Clarify ($Pb(NO_3)_2$)
		Adsorb on zeolite	Method	
				Filtrate
		Eluate (acid KCl)		
		Thiochrome		$CNBr\text{-}PhNH_2$
		Method		Method

Express results on "as received," dry, and "35 per cent moisture" basis.

The small difference between the one and 2 gram sample, seen in this case, is not consistently observed. One gram samples, especially of non-enriched bread, approach the lower limits of sensitivity of the method, hence, 2 gram samples are preferred.

Because of the ease of extracting free thiamine from the simple mixture constituting enriched flour, it is expedient for control purposes simply to shake the sample with the potassium chloride solution and, omitting the phosphatase procedure and the zeolite adsorption, test the salt extract directly. The enzyme treatment, however, is necessary in the case of bread since yeast during active fermentation converts free thiamine to the phosphorylated vitamin.

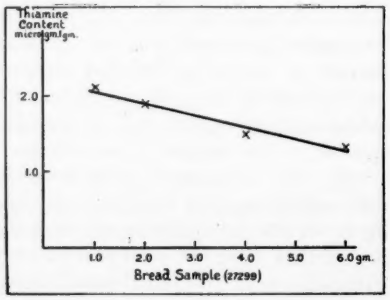


FIG. 2

form available for the microorganism. This nicotinic acid precursor is not found in animal products. It is not nicotinamide, Co-enzyme I or II, or any other known derivative of nicotinic acid. Whether or not it is biologically active for the human has yet to be determined. Therefore, justification for the higher values obtained on the hydrolyzed extracts must await the results of further studies. The chemical procedure has the advantage of greater speed with respect to the overall time required to obtain results and to the number of analyses an individual can conduct within a given time.

The results of bread analyses should be expressed in terms of a one-pound loaf on the "as received," dry and 35 per cent moisture basis. Since no loss of thiamine occurs during air-drying, the relative thiamine content per pound of loaf increases with the staleness of the bread. By expressing the results on the dry basis, comparisons can be made from time to time on any particular product regardless of the staleness of the sample when received or of variations in dehydration during baking. It is also desirable to express results in terms of bread containing 35 per cent moisture, since this is the average value for freshly baked bread. The conventional 38 per cent moisture value is a maximal figure and is rarely observed in practice.

The principal methods for enriching bread commercially are the use of a high-vitamin yeast or of a compressed tablet containing the appropriate amounts of vitamins and minerals for the conventional size dough batch. Control tests of the tablets offer no unusual difficulties since the vitamins are present entirely in free synthetic form. In the case of high-vitamin yeast the thiamine is present principally in the phosphorylated state which necessitates the enzymic hydrolysis, if either of the chemical methods is to be used in the analysis. The yeast fermentation method involves measurement of the stimulating effect of thiamine upon carbon dioxide production by a particular strain of yeast in a specially devised fermentometer. Inexpensive improvisations of this equipment have been described. In the fermentation method no distinction is made between free and phosphorylated thiamine. Correction must be made for the stimulatory effect of pyrimidine compounds.

The numerous technical and legal problems surrounding the production and control of vitamin D milk have been effectively solved so that this food can now be had almost anywhere in the country. The methods for controlling vitamin D milk depend on which of the three major processes is used. This is illustrated in table 6. It is well known that cows fed large doses of vitamin D

TABLE 6

Control of vitamin D milk

Milk

Activated by

	Metabolic process	*Irradiation*	*Addition*
PLANT CONTROLS	Bioassay Irradiated Yeast.	U. V. Energy Input.	Bioassays of concentrate
	Intake of D vs. Output of Milk.	Rate of Flow.	Production records—Concentrate used vs. Milk produced.
	Herd records.	Thickness of Film.	
	400–430 u./qt.	135 u./qt.	400 u./qt.
MARKET CONTROLS	*Bioassays of market samples* Reported to Dairy; Health Officer; Licensor; A.M.A.		

secrete a small proportion (about 2 or 3 per cent) of the vitamin in the milk. Of the various forms of vitamin D, irradiated yeast is the only one that is commercially employed to any extent for the commercial production of vitamin D milk. Control of this so-called "metabolized vitamin D" milk necessitates the maintenance of careful records of the vitamin D consumption and milk output of the herd. These records are kept available for inspection and copies are submitted to the licensor who supplies the irradiated yeast. By this method milk of approximately 430 U.S.P. units per quart is produced.

Antirachitic activation of milk by exposure to the radiation of a carbon or mercury arc has been intensively studied under the auspices of the Wisconsin Alumni Research Foundation. Production control is concerned principally with physical and engineering phases. Milk irradiators are equipped with control devices which

regulate the intensity of the ultraviolet radiation, and rate of flow of the milk. The velocity and thickness of the film, and the time, distance, intensity and quantity of radiation have been carefully standardized with the view toward producing milk of uniform potency, namely, 135 U.S.P. units per quart.

To facilitate fortification of milk by the direct addition of vitamin D, the activated sterol or fish liver oil preparation is furnished in the form of an emulsion in cream. This is packed in cans on which the label states the volume of milk to be fortified by the contents of each can. Dairy operators are instructed in the procedure for proper two-stage mixing of the concentrate. A special graduate is also furnished to provide for dilution of graded portions of the concentrate when less than a full can is used.

In certain states and municipalities the dairy is required by law to furnish the local milk inspector or other health official with copies of assays of samples of vitamin D milk collected by the inspector or purchased without previous notice by an approved independent laboratory. Licensors of irradiation and fortification processes also make it a condition of their agreements that such assays be made by or submitted to them.

When samples are received in the iced milk shippers, it is our practice immediately to unpack and make a record of the sample and to file the hood of the bottle. One-tenth of a cubic centimeter of 10 per cent formaldehyde is added, the milk thoroughly mixed and refrigerated. The milk is mixed each day until the completion of the test in order to avoid creaming since it is occasionally necessary to repeat the assay. In the A.O.A.C. method it is stated that soured or curdled milk is unsuitable for assay purposes. However, we have had occasion to neutralize sour milk with sodium bicarbonate and re-emulsify, and we have obtained satisfactory assays in such cases.

The A.O.A.C. procedure for assay of vitamin D milk is patterned after the U.S.P. method. Several optional methods are offered for feeding the milk and the assay period may be either 7 or 10 days. It is also provided that an amount of ether extracted skim milk powder equal in weight to the solids-not-fat (9 per cent) of the daily dose of assay milk, be fed to the control animals receiving the U.S.P. Reference Cod Liver Oil. It is of interest to note that while the A.O.A.C. method, like the U.S.P. vitamin assay procedures, does not provide for a tolerance in the interpretation of the line test responses, the New York State Department of Health has recently announced a tolerance of 0.2 in rating the line test diagnosis on the Bills scale.

REFERENCES

ANDREWS, J. S. Collaborative study of the chemical and microbiological methods for determination of riboflavin in cereal products. Cereal Chem., In Press (1942). ANDREWS, J. S., AND NORDGREN, R. The application of the thiochrome method to the thiamin analysis of cereals and cereal products. Cereal Chem. **18:** 686 (1941). ANDREWS, J. S., BOYD, H. M., AND TERRY, D. E. Riboflavin analysis of cereals. Application of the microbiological method. Anal. Ed., Ind. Eng. Chem. **14:** 271 (1942). BESSEY, O. A. A method for the determination of small quantities of ascorbic acid and dehydroascorbic acid in turbid and colored solutions in the presence of other reducing substances. J. Biol. Chem. **126:** 771 (1938). BESSEY, O. A., AND KING, C. G. The distribution of vitamin C in plant and animal tissues, and its determination. J. Biol. Chem. **103:** 687 (1933). BIRCH, T. W. The requirements of the dog and the rat for nicotinic acid. J. Nutrition **17:** 281 (1939). BOOHER, L. E. The vitamin A values of 128 foods as determined by the rat growth method. U. S. Dept. of Agriculture, Technical Bulletin No. 802 (1941). COWARD, K., AND KASSNER, E. W. The determination of vitamin C by means of its influence on the body weight of guinea pigs. Biochem J. **30:** 1719 (1936). DANN, W. J., AND EVELYN, K. A. The determination of vitamin A with the photoelectric colorimeter. Biochem. J. **32:** 1008 (1938). DORFMAN, A., KOSER, STEWART A., HORWITT, M. K., BERKMAN, S., AND SAUNDERS, F. Quantitative response of the dysentery bacillus to nicotinamide and related compounds. Proc. Soc. Exp. Bio. Med. **43:** 434 (1940). ELVEHJEM, C. A., MADDEN, R. J., STRONG, F. M., AND WOOLLEY, D. W. Relation of nicotinic acid and nicotinic acid amide to canine blacktongue. J. Am. Chem. Soc. **59:** 1767 (1937). EVELYN, K. A., MALLOY, H. A., AND ROSEN, C. The determination of ascorbic acid in urine with the photoelectric colorimeter. J. Biol. Chem. **126:** 645 (1938). Hearings on the Enrichment of Macaroni. Federal Security Agency, Washington, D. C. (1941). GOLDBERGER, J., WHEELER, G. A., LILLIE, R. D., AND ROGERS, L. M. Experimental black tongue and the blacktongue preventive in yeast. U. S. Public Health Rep. 43, 657 (1928). HENNESSY, D. J. Chemical methods for determination of vitamin B_1. Anal. Ed., Ind. Eng. Chem. **14:** 35 (1942). HODSON, A. Z., AND NORRIS, L. C. A fluorometric method for determining the riboflavin content of foodstuffs. J. Biol. Chem. **131:** 621 (1939). HÖJER, J. A. Method for determining

the antiscorbutic value of foodstuffs by means of histologic examination of the teeth of young guinea pigs. Brit. J. Exp. Path. **7:** 356 (1926). HORWITT, M. K., COWGILL, G. R., AND MENDEL, L. B. The availability of the proteins and inorganic salts of the green leaf. J. Nutrition **12:** 237 (1936). HORWITT, M. K., COWGILL, G. R., AND MENDEL, L. B. The availability of the carbohydrates and fats of the green leaf together with some observations on crude fiber. J. Nutrition **12:** 255 (1936). KING, C. G. Chemical methods for determination of vitamin C. Anal. Ed., Ind. Eng. Chem. **13:** 225 (1941). KLINE, O. L., HALL, W. L., AND MORGAN, J. F. An improved rat growth method for the assay of vitamin B_1, including sulfite treatment of dietary constituents. J. Assoc. Off. Agr. Chem. **24:** 147 (1941). KINNERSLEY, H. W., PETERS, R. A., AND READER, V. A quantitative comparison of the curative activity of torulin (vitamin B_1) upon the adult pigeon and the adult white rat. Biochem. J. **24:** 1820 (1930). LOOFBOUROW, J. R., AND HARRIS, R. H. The evaluation of fluorophotometers to be used in the thiochrome assay for vitamin B_1. Cereal Chem. **19:** 151 (1942). MEIKLEJOHN, A. P. The estimation of vitamin B_1 in blood by a modification of Schopfer's test. Biochem. J. **31:** 1441 (1937). MELNICK, D. Collaborative study of the applicability of microbiological and chemical methods to the determination of niacin (nicotinic acid) in cereal products. Cereal Chem., In Press (1942). MELNICK, D., AND FIELD, H., JR. Chemical determination of vitamin B_1. Reaction between thiamine and diazotized p-aminoacetophenone. J. Biol. Chem. **127:** 505, 515, 531 (1939). MELNICK, D., MABARDIE, A., BERNSTEIN, A., AND OSER, B. L. The fate of vitamin B_1 in the production of baked goods. Northwestern Miller **208:** 17 (1941). MELNICK, D., OSER, B. L., AND SIEGEL, L. Chemical determination of nicotinic acid content of flour and bread. Anal. Ed. Ind. Eng. Chem. **13:** 879 (1941). MILAS, N. A., HEGGIE, R., RAYNOLDS, J. A. A spectroscopic method for the quantitative estimation of vitamin D. Anal. Ed., Ind. Eng. Chem. **13:** 227 (1941). MINDLIN, R. L., BUTLER, ALLAN M. The determination of ascorbic acid in plasma, a macromethod and micromethod. J. Biol. Chem. **122:** 673 (1938). MORGAREIDGE, K. Influence of solvent on the ultraviolet absorption curve of vitamin A. Am. Soc. Biol. Chem., Federation Proc. **1:** 127 (1942). MUSULIN, R. R., AND KING, C. G. Metaphosphoric acid in the extraction and titration of vitamin C. J. Biol. Chem. **116:** 409 (1936). NEAL, R. H., HAURAND, C. H., AND LUCKMANN, F. H. Determination of total vitamin A content of dairy butters. (Spectrophotometric method.) Anal. Ed., Ind. Eng. Chem. **13:** 150 (1941). Official and Tentative Methods of Analysis. Assn. Off. Agr. Chem., (1940). OSER, B. L. The laboratory control of enriched foods. Federation Proceedings, In Press (1942). PARSONS, H. T., COLLARD, J., GARDNER, J., STRONG, F. M., PETERSON, W. H. Utilization by man of the vitamin B complex in fresh yeast. Am. Soc. Biol. Chem., Federation Proc. **1:** 129 (1942). PETERSON, W. J. Recent developments in methods for determining carotene. Anal. Ed., Ind. Eng. Chem. **13:** 212 (1941). ROUTH, J. I. AND HOUCHIN, O. B. Some nutritional requirements of the hamster. Am. Inst. Nutrition, Federation Proc. **1:** 191 (1942). SCHULTZ, A. S., ATKIN, L. AND FREY, C. N. Determination of vitamin B_1 by yeast fermentation method. Anal. Ed., Ind. Eng. Chem. **14:** 35 (1942). SNELL, E. E. AND STRONG, F. M. A microbiological assay of riboflavin. Anal. Ed., Ind. Eng. Chem. **11:** 346 (1939). SNELL, E. E. AND WRIGHT, L. D. A microbiological method for the determination of nicotinic acid. J. Biol. Chem. **139:** 675 (1941). SHERMAN-BOURQUIN. Method for the Bioassay of Vitamin G Cited in SHERMAN, H. C. AND SMITH, S. L. The vitamins. Chemical Publishing Co., 2nd edition (1931). STEVENS, J. W. Estimation of ascorbic acid in citrus juices by iodine titration method. Anal. Ed., Ind. Eng, Chem. **10:** 269 (1938). Studies on the vitamin content of tissues. I. University of Texas Publication No. 4137 (1941). U. S. Pharmacopoeia, Eleventh Revision, Second Supplement (1939). WILKIE, J. B. Physical and chemical determination of vitamin A. Anal. Ed., Ind. Eng. Chem. **13:** 209 (1941).

FEDERATION OF AMERICAN SOCIETIES FOR EXPERIMENTAL BIOLOGY

SPECIAL NOTICE IN RELATION TO THE 1943 MEETING

In October 1942 the Federation Executive Committee, cognizant of the numerous problems connected with the war effort, voted, a vote which was subsequently ratified by the Councils of all the constituent societies:

> To omit the annual meeting of the Federation in 1943.

The Federation Executive Committee at the same time also voted:

> To publish a second (1943) volume of the Federation Proceedings patterned after the first volume.

This vote:

(a) Does not preclude separate meetings of the individual Societies, and

(b) Does provide for the publication of abstracts and symposium papers which would have been presented if a Federation annual meeting were to be held or which will be presented if individual societies hold separate meetings in Cleveland or elsewhere at or about the time the Federation meeting was scheduled, i.e., April 6–10, 1943.

It is thus intended that intercourse will be assured to the workers in the biological sciences insofar as is compatible with a total war effort.

EXECUTIVE COMMITTEE, 1942

PHILIP BARD, WALLACE O. FENN, The Physiological Society

RUDOLPH J. ANDERSON, ARNOLD K. BALLS, The Biochemical Society

E. K. MARSHALL, JR., RAYMOND N. BIETER, The Pharmacological Society

BALDUIN LUCKÉ, H. P. SMITH, The Pathological Society

LEONARD A. MAYNARD, ARTHUR H. SMITH, The Institute of Nutrition

JACQUES J. BRONFENBRENNER, ARTHUR F. COCA, The Association of Immunologists

PHILIP BARD, *Chairman*, Johns Hopkins Medical School, Baltimore, Md.

ALBERT G. HOGAN, *Ex-Chairman*

D. R. HOOKER, *Secretary*, 19 W. Chase St., Baltimore, Md.

STANDING COMMITTEES

Defence of Biological Research: ELLIOTT C. CUTLER, *Chairman;* A. B. LUCKHARDT, C. I. REED, G. H. WHIPPLE.

International Congresses: H. S. GASSER, Physiology, *Chairman;* A. J. CARLSON, Physiology; D. D. VAN SLYKE, Biochemistry; E. K. MARSHALL, JR., Pharmacology; PEYTON ROUS, Pathology; L. A. MAYNARD, Nutrition.

Public Information: HARRY GOLDBLATT, *Chairman;* R. G. HOSKINS, R. W. GERARD.

Placement Service: H. B. LEWIS, *Director.*

Representatives, Council A.A.A.S.: G. PHILIP GRABFIELD, CHARLES G. KING.

Federation Proceedings, Control Committee: PHILIP BARD, *Chairman;* C. G. KING, MORTON MCCUTCHEON, C. F. SCHMIDT, A. H. SMITH.

FORMER EXECUTIVE COMMITTEES

Philadelphia, Dec. 28–31, 1913

S. J. MELTZER, *Chairman*, and A. J. CARLSON, *Secretary*, The Physiological Society. A. B. MACALLUM and P. A. SHAFFER, The Biochemical Society. T. SOLLMANN and J. AUER, The Pharmacological Society.

St. Louis, Dec. 27–30, 1914

G. LUSK, *Chairman*, and P. A. SHAFFER, *Secretary*, The Biochemical Society. T. SOLLMANN and J. AUER, The Pharmacological Society. R. M. PEARCE and G. H. WHIPPLE, The Pathological Society. W. B. CANNON and A. J. CARLSON, The Physiological Society.

Boston, Dec. 26–29, 1915

TORALD SOLLMANN, *Chairman*, and JOHN AUER, *Secretary*, The Pharmacological Society. THEOBALD SMITH and PEYTON ROUS, The Pathological Society. W. B. CANNON and C. W. GREENE, The Physiological Society. WALTER JONES and P. A. SHAFFER, The Biochemical Society.

B & Z Library

New York, Dec. 27–30, 1916

SIMON FLEXNER, *Chairman*, and PEYTON ROUS, *Secretary*, The Pathological Society. W. B. CANNON and C. W. GREENE, The Physiological Society. WALTER JONES and STANLEY R. BENEDICT, The Biochemical Society. REID HUNT and J. AUER, The Pharmacological Society.

Minneapolis-Rochester, Dec. 27–29, 1917

FREDERIC S. LEE, *Chairman*, and CHARLES W. GREENE, *Secretary*, The Physiological Society. CARL L. ALSBERG and STANLEY R. BENEDICT, The Biochemical Society. REID HUNT and L. G. ROWNTREE, The Pharmacological Society. LUDVIG HEKTOEN and HOWARD T. KARSNER, The Pathological Society.

Baltimore, April 24–26, 1918

CARL L. ALSBERG, *Chairman*, and STANLEY R. BENEDICT, *Secretary*, The Biochemical Society. REID HUNT and E. D. BROWN, The Pharmacological Society. H. GIDEON WELLS and HOWARD T. KARSNER, The Pathological Society. FREDERIC S. LEE and CHARLES W. GREENE, The Physiological Society.

Cincinnati, Dec. 29–31, 1919

A. S. LOEVENHART, *Chairman*, and E. D. BROWN, *Secretary*, The Pharmacological Society. W. G. MACCALLUM and HOWARD T. KARSNER, The Pathological Society. WARREN P. LOMBARD and CHARLES W. GREENE, The Physiological Society. STANLEY R. BENEDICT and VICTOR C. MYERS, The Biochemical Society.

Chicago, Dec. 28–30, 1920

WILLIAM H. PARK, *Chairman*, and HOWARD T. KARSNER, *Secretary*, The Pathological Society. WARREN P. LOMBARD and CHARLES W. GREENE, The Physiological Society. STANLEY R. BENEDICT and VICTOR C. MYERS, The Biochemical Society. A. S. LOEVENHART and EDGAR D. BROWN, The Pharmacological Society.

New Haven, Dec. 28–30, 1921

J. J. R. MACLEOD, *Chairman*, and CHARLES W. GREENE, *Secretary*, The Physiological Society. D. D. VAN SLYKE and VICTOR C. MYERS, The Biochemical Society. C. W. EDMUNDS and EDGAR D. BROWN, The Pharmacological Society. F. G. NOVY and WADE H. BROWN, The Pathological Society.

Toronto, Dec. 27–29, 1922

D. D. VAN SLYKE, *Chairman*, and VICTOR C. MYERS, *Secretary*, The Biochemical Society. C. W. EDMUNDS and EDGAR D. BROWN, The Pharmacological Society. HOWARD T. KARSNER and WADE H. BROWN, The Pathological Society. J. J. R. MACLEOD and CHARLES W. GREENE, The Physiological Society.

St. Louis, Dec. 27–29, 1923

C. W. EDMUNDS, *Chairman*, and EDGAR D. BROWN, *Secretary*, The Pharmacological Society. E. L. OPIE and WADE H. BROWN, The Pathological Society. A. J. CARLSON and CHARLES W. GREENE, The Physiological Society. PHILIP A. SHAFFER and VICTOR C. MYERS, The Biochemical Society.

Washington, Dec. 29–31, 1924

ALDRED S. WARTHIN, *Chairman*, and E. B. KRUMBHAAR, *Secretary*, The Pathological Society. A. J. CARLSON and WALTER J. MEEK, The Physiological Society. P. A. SHAFFER and D. WRIGHT WILSON, The Biochemical Society. JOHN AUER and E. D. BROWN, The Pharmacological Society.

Cleveland, Dec. 28–30, 1925

A. J. CARLSON, *Chairman*, and WALTER J. MEEK, *Secretary*, The Physiological Society. H. C. SHERMAN and D. WRIGHT WILSON, The Biochemical Society. JOHN AUER and E. D. BROWN, The Pharmacological Society. GEORGE H. WHIPPLE and E. B. KRUMBHAAR, The Pathological Society.

Rochester, N. Y., April 14–16, 1927

E. C. KENDALL, *Chairman*, and F. C. KOCH, *Secretary*, The Biochemical Society. JOHN AUER and E. D. BROWN, The Pharmacological Society. W. H. BROWN and E. B. KRUMBHAAR, The Pathological Society. J. ERLANGER and W. J. MEEK, The Physiological Society.

Ann Arbor, April 12–14, 1928

CARL VOEGTLIN, *Chairman*, and E. D. BROWN, *Secretary*, The Pharmacological Society. DAVID MARINE and CARL V. WELLER, The Pathological Society. JOSEPH ERLANGER and WALTER J. MEEK, The Physiological Society. E. V. MCCOLLUM and D. WRIGHT WILSON, The Biochemical Society.

Boston, Aug. 19–24, 1929

(The XIIIth International Physiological Congress)

EDWARD B. KRUMBHAAR, *Chairman*, and CARL V. WELLER, *Secretary*, The Pathological Society. JOSEPH ERLANGER and WALTER J. MEEK, The Physiological Society. E. V. MCCOLLUM and D. WRIGHT WILSON, The Biochemical Society. CARL VOEGTLIN and E. D. BROWN, The Pharmacological Society.

Chicago, March 26–29, 1930

WALTER J. MEEK, *Chairman*, and ALFRED C. REDFIELD, *Secretary*, The Physiological Society.

W. R. BLOOR and HOWARD B. LEWIS, The Biochemical Society. CARL VOEGTLIN and E. D. BROWN, The Pharmacological Society. WILLIAM F. PETERSEN and CARL V. WELLER, The Pathological Society.

Montreal, April 8–11, 1931

W. R. BLOOR, *Chairman*, and H. B. LEWIS, *Secretary*, The Biochemical Society. GEORGE B. WALLACE and E. D. BROWN, The Pharmacological Society. FREDERICK L. GATES and C. PHILLIP MILLER, The Pathological Society. WALTER J. MEEK and ARNO B. LUCKHARDT, The Physiological Society.

Philadelphia, April 27–30, 1932

GEORGE B. WALLACE, *Chairman*, and V. E. HENDERSON, *Secretary*, The Pharmacological Society. SAMUEL R. HAYTHORN and C. PHILLIP MILLER, The Pathological Society. WALTER J. MEEK and ARNO B. LUCKHARDT, The Physiological Society. H. C. BRADLEY and HOWARD B. LEWIS, The Biochemical Society.

Cincinnati, April 10–12, 1933

PEYTON ROUS, *Chairman*, and C. PHILLIP MILLER, *Secretary*, The Pathological Society. ARNO B. LUCKHARDT and FRANK C. MANN, The Physiological Society. H. C. BRADLEY and HOWARD B. LEWIS, The Biochemical Society. WM. DEB. MACNIDER and V. E. HENDERSON, The Pharmacological Society.

New York, March 28–31, 1934

ARNO B. LUCKHARDT, *Chairman*, FRANK C. MANN, *Secretary*, and ALEXANDER FORBES, *Treasurer*, The Physiological Society. W. M. CLARK and H. A. MATTILL, The Biochemical Society. W. DEB. MACNIDER and V. E. HENDERSON, The Pharmacological Society. CARL V. WELLER and C. PHILLIP MILLER, The Pathological Society.

Detroit, April 10–13, 1935

W. M. CLARK, *Chairman*, H. A. MATTILL, *Secretary*, and C. H. FISKE, *Treasurer*, the Biochemical Society. CHARLES W. GREENE and FRANK C. MANN, The Physiological Society. R. A. HATCHER and E. M. K. GEILING, The Pharmacological Society. S. BURT WOLBACH and SHIELDS WARREN, The Pathological Society.

Washington, March 25–28, 1936

V. E. HENDERSON, *Chairman*, E. M. K. GEILING, *Secretary*, and C. M. GRUBER, *Treasurer*, The Pharmacological Society. FRANK C. MANN and ANDREW C. IVY, The Physiological Society. H. B. LEWIS and H. A. MATTILL, The Biochemical Society. OSKAR KLOTZ and SHIELDS WARREN, The Pathological Society.

Memphis, April 21–24, 1937

ALPHONSE R. DOCHEZ, *Chairman*, and SHIELDS WARREN, The Pathological Society. FRANK C. MANN and ANDREW C. IVY, The Physiological Society. HOWARD B. LEWIS and H. A. MATTILL, The Biochemical Society. V. E. HENDERSON and E. M. K. GEILING, The Pharmacological Society. D. R. HOOKER, *Secretary*.

Baltimore, March 30–April 2, 1938

WILLIAM T. PORTER, *Honorary President*; WALTER E. GARREY, *Chairman*, and ANDREW C. IVY, The Physiological Society. GLENN E. CULLEN and H. A. MATTILL, The Biochemical Society. ARTHUR L. TATUM and G. PHILIP GRABFIELD, The Pharmacological Society. C. PHILLIP MILLER and PAUL R. CANNON, The Pathological Society. D. R. HOOKER, *Secretary*.

Toronto, April 26–29, 1939

GLENN E. CULLEN, *Chairman*, and CHARLES G. KING, The Biochemical Society. ARTHUR L. TATUM and G. PHILIP GRABFIELD, The Pharmacological Society. C. PHILLIP MILLER and PAUL R. CANNON, The Pathological Society. WALTER E. GARREY and ANDREW C. IVY, The Physiological Society. D. R. HOOKER, *Secretary*.

New Orleans, March 13–16, 1940

E. M. K. GEILING, *Chairman*, and G. PHILIP GRABFIELD, The Pharmacological Society. ERNEST W. GOODPASTURE and PAUL R. CANNON, The Pathological Society. ANDREW C. IVY and PHILIP BARD, The Physiological Society. WILLIAM C. ROSE and CHARLES G. KING, The Biochemical Society. D. R. HOOKER, *Secretary*.

Chicago, April 15–19, 1941

SHIELDS WARREN, *Chairman*, and H. P. SMITH, The Pathological Society. THORNE M. CARPENTER and L. A. MAYNARD, The Institute of Nutrition. ANDREW C. IVY and PHILIP BARD, The Physiological Society. WILLIAM C. ROSE and CHARLES G. KING, The Biochemical Society. E. M. K. GEILING and G. PHILIP GRABFIELD, The Pharmacological Society. D. R. HOOKER, *Secretary*.

Boston, March 31, April 1, 2, 3, 4, 1942

ALBERT G. HOGAN, *Chairman*, and ARTHUR H. SMITH, The Institute of Nutrition. PHILIP BARD and CARL J. WIGGERS, The Physiological Society. RUDOLPH J. ANDERSON and ARNOLD K. BALLS, The Biochemical Society. E. M. K. GEILING and R. N. BIETER, The Pharmacological Society. JESSE L. BOLLMAN and H. P. SMITH, The Pathological Society. SHIELDS WARREN, *Ex-Chairman*. D. R. HOOKER, *Secretary*.

BY-LAWS

BY-LAWS

Adopted at the Washington Meeting, 1936

1. The Presidents and Secretaries of the Constituent Societies, the Chairman of the Executive Committee of the preceding year and the Federation Secretary shall form the Executive Committee of the Federation.

2. The Chairmanship of the Executive Committee shall be held in turn by the Presidents of the Constituent Societies, who shall succeed one another annually in the order of seniority of the Societies.

3. The Executive Committee shall appoint annually from the membership of the Federation a secretary-treasurer, to be known as the Federation Secretary.

4. The Federation Secretary shall: (a) Keep the minutes of the Executive Committee and distribute copies to the Secretaries of the Constituent Societies. (b) Make arrangements for the Annual Meeting with the Local Committee, with the approval of the Executive Committee. (c) Print in convenient combined form and distribute to the membership of the Federation the programs of the Constituent Societies as received from their respective Secretaries. (d) Undertake such other duties, to be decided upon from time to time by the Executive Committee, as do not conflict with the complete autonomy of the Constituent Societies.

5. The Executive Committee shall control all monies in the hands of the Federation Secretary, who shall make an annual report to the Executive Committee for audit and approval. The expenses of the Federation Secretary, as authorized by the Executive Committee, shall be the first charge on such monies and if insufficient for the purpose the Executive Committee shall prorate such expenses to the Constituent Societies of the Federation in proportion to their respective memberships.

The Executive Committee may appropriate Federation monies annually for the uses of Local Committees and for the uses of other authorized Committees but in the latter cases an audit of expenditures shall be made and approved before such committees are discharged.

6. The Executive Committee shall determine the place of the Annual Meeting, and the time shall be determined by the Local Committee, preferably within the period of March fifteenth to May first.

7. The local Committee at the place of meeting of the Federation shall charge such fee for registration as may be approved by the Executive Committee. The monies thus collected shall be used to defray the expenses of the Local Committee and the remainder, after such expenses have been met, shall be turned over to the Federation Secretary.

8. The Executive Committee shall consider measures of advantage to the Federation as a whole. Any Constituent Society may refer similar measures to the Executive Committee. No action, however, shall be taken by the Executive Committee unless specifically authorized by all the Constituent Societies.

9. The Chairman of the Executive Committee may appoint committees when the purposes of such committees have been approved by all the Constituent Societies of the Federation. Such committees shall be appointed for a term of one year, but may be continued and their members reappointed. Such committees shall report in writing to the Executive Committee, which shall in turn report thereon to the Constituent Societies either for information or recommendation. The Secretaries of the Constituent Societies shall report the recommendations of their respective Societies to the Executive Committee for final action.

10. All individuals whose names appear on the program by invitation or introduction and those registering from any recognized biological laboratory may be enrolled as Associate Members of the Federation for that Annual Meeting. Such Associate Members may enjoy all the privileges of the Annual Meeting except that of voting.

11. No person may present orally more than one paper during all of the scientific sessions of the Constituent Societies at the time of the Annual Meeting except upon invitation of the Executive Committee or a Council. Papers must be submitted to the Secretary of the Society of which the proposer is a member. The proposer may request transfer to another program, but this may only be done with the consent of the Secretary of the Society concerned. Any Secretary who regards any paper submitted to him as better suited to the program of another Society may arrange this transfer with the Secretary of the Society concerned, if it be possible. Such transfer shall be indicated on the program.

12. Abstracts not to exceed two hundred and fifty words in length, of papers approved for presentation at all of the scientific sessions of all the Constituent Societies at the Annual Meeting, shall receive publication in the *Federation Proceedings*.

13. A Control Committee, consisting of at least one representative of each Constituent Society as designated by the several Councils, shall have editorial control over the *Federation Proceedings* which shall be financed as required by an annual assessment of all the members of each Constituent Society.

14. The Control Committee shall have power to choose certain additional papers presented at the Annual Meetings and from other sources, including material heretofore published in the Federation Yearbook, for publication in the Federation Proceedings.

PLACEMENT SERVICE

The Federation maintains a service to act as a medium of communication between persons seeking positions for teaching or research and institutions that wish to fill vacancies in these sciences.

The service does not undertake to recommend or to pass judgment upon applicants. It aims merely to serve as a clearing-house for such information as above stated and to bring into touch with one another candidates for positions and vacancies to be filled.

Persons, whether members of the Federation or not, and institutions desiring to avail themselves of the service, may receive such information as is available without cost to the applicant.

All communications should be addressed to Dr. H. B. Lewis, Director, University of Michigan, Ann Arbor, Mich.

THE AMERICAN PHYSIOLOGICAL SOCIETY

Founded December 30, 1887; Incorporated June 2, 1923

OFFICERS ELECTED 1942

President—PHILIP BARD, Johns Hopkins School of Medicine, Baltimore, Md.

Secretary—WALLACE O. FENN, University of Rochester School of Medicine and Dentistry, Rochester, N. Y.

Treasurer—HALLOWELL DAVIS, Harvard University School of Medicine, Boston, Mass.

Council—PHILIP BARD, WALLACE O. FENN, HALLOWELL DAVIS, CHARLES H. BEST, University of Toronto, Canada, MAURICE B. VISSCHER, University of Minnesota, Minneapolis, HIRAM E. ESSEX, Mayo Foundation, Rochester, Minn., W. F. HAMILTON, University of Georgia, Augusta.

Board of Publication Trustees—WALTER J. MEEK, HENRY C. BAZETT, ANDREW C. IVY.

PAST OFFICERS

Organization Meeting, December 30, 1887

S. WEIR MITCHELL, *President*
H. N. MARTIN, *Secretary*

1888 H. P. BOWDITCH, President; H. N. MARTIN, Secretary-Treasurer; J. G. CURTIS, H. C. WOOD, H. SEWALL, Councilors. **1889** S. WEIR MITCHELL, President; H. N. MARTIN, Secretary-Treasurer; H. P. BOWDITCH, J. G. CURTIS, H. C. WOODS, Councilors. **1890** S. WEIR MITCHELL, President; H. N. MARTIN, Secretary-Treasurer; H. P. BOWDITCH, J. G. CURTIS, H. H. DONALDSON, Councilors. **1891** H. P. BOWDITCH, President; H. N. MARTIN, Secretary-Treasurer; R. H. CHITTENDEN, J. G. CURTIS, H. H. DONALDSON, Councilors. **1892** H. P. BOWDITCH, President; H. N. MARTIN, Secretary-Treasurer; R. H. CHITTENDEN, J. G. CURTIS, W. H. HOWELL, Councilors. **1893** H. P. BOWDITCH, President; W. P. LOMBARD, Secretary-Treasurer; R. H. CHITTENDEN, J. G. CURTIS, W. H. HOWELL, Councilors. **1894** H. P. BOWDITCH, President; W. P. LOMBARD, Secretary-Treasurer; R. H. CHITTENDEN, W. H. HOWELL, J. W. WARREN, Councilors. **1895** H. P. BOWDITCH, President; F. S. LEE, Secretary-Treasurer; R. H. CHITTENDEN, W. H. HOWELL, W. P. LOMBARD, Councilors. **1896** R. H. CHITTENDEN, President; F. S. LEE, Secretary-Treasurer; H. P. BOWDITCH, W. H. HOWELL, J. W. WARREN, Councilors. **1897** R. H. CHITTENDEN, President; F. S. LEE, Secretary-Treasurer; H. P. BOWDITCH, W. H. HOWELL, W. P. LOMBARD, Councilors. **1898** R. H. CHITTENDEN, President; F. S. LEE, Secretary-Treasurer; H. P. BOWDITCH, W. H. HOWELL, W. P. LOMBARD, Councilors. **1899** R. H. CHITTENDEN, President; F. S. LEE, Secretary-Treasurer; W. H. HOWELL, W. P. LOMBARD, W. T. PORTER, Councilors. **1900** R. H. CHITTENDEN, President; F. S. LEE, Secretary-Treasurer; W. H. HOWELL, W. P. LOMBARD, W. T. PORTER, Councilors. **1901** R. H. CHITTENDEN, President; F. S. LEE, Secretary-Treasurer; W. H. HOWELL, W. P. LOMBARD, W. T. PORTER, Councilors. **1902** R. H. CHITTENDEN, President; F. S. LEE, Secretary-Treasurer; W. H. HOWELL, W. P. LOMBARD, W. T. PORTER, Councilors. **1903** R. H. CHITTENDEN, President; F. S. LEE, Secretary-Treasurer; W. H. HOWELL, W. P. LOMBARD, W. T. PORTER, Councilors. **1904** R. H. CHITTENDEN, President; W. T. PORTER, Secretary-Treasurer; F. S. LEE, W. P. LOMBARD, W. H. HOWELL, Councilors. **1905** W. H. HOWELL, President; L. B. MENDEL, Secretary; W. B. CANNON, Treasurer; R. H. CHITTENDEN, S. J. MELTZER, Councilors. **1906** W. H. HOWELL, President; L. B. MENDEL, Secretary; W. B. CANNON, Treasurer; A. B. MACALLUM, S. J. MELTZER, Councilors. **1907** W. H. HOWELL, President; L. B. MENDEL, Secretary; W. B. CANNON, Treasurer; J. J. ABEL, G. LUSK, Councilors. **1908** W. H. HOWELL, President; R. HUNT, Secretary; W. B. CANNON, Treasurer; J. J. ABEL, G. LUSK, Councilors. **1909**

W. H. Howell, President; R. Hunt, Secretary; W. B. Cannon, Treasurer; A. J. Carlson, W. P. Lombard, Councilors. **1910** W. H. Howell, President; A. J. Carlson, Secretary; W. B. Cannon, Treasurer; J. Erlanger, F. S. Lee, Councilors. **1911** S. J. Meltzer, President; A. J. Carlson, Secretary; W. B. Cannon, Treasurer; J. Erlanger, F. S. Lee, Councilors. **1912** S. J. Meltzer, President; A. J. Carlson, Secretary; W. B. Cannon, Treasurer; J. Erlanger, F. S. Lee, Councilors. **1913** S. J. Meltzer, President; A. J. Carlson, Secretary; J. Erlanger, Treasurer; W. B. Cannon, F. S. Lee, Councilors. **1914** W. B. Cannon, President; A. J. Carlson, Secretary; J. Erlanger, Treasurer; F. S. Lee, S. J. Meltzer, Councilors. **1915** W. B. Cannon, President; C. W. Greene, Secretary; J. Erlanger, Treasurer; W. E. Garrey, W. H. Howell, J. J. R. Macleod, W. J. Meek, Councilors. **1916** W. B. Cannon, President; C. W. Greene, Secretary; J. Erlanger, Treasurer; W. E. Garrey, W. H. Howell, J. J. R. Macleod, W. J. Meek, Councilors. **1917** F. S. Lee, President; C. W. Greene, Secretary; J. Erlanger, Treasurer; W. B. Cannon, W. H. Howell, J. J. R. Macleod, W. J. Meek, Councilors. **1918** F. S. Lee, President; C. W. Greene, Secretary; J. Erlanger, Treasurer; W. B. Cannon, W. H. Howell, J. J. R. Macleod, W. J. Meek, Councilors. **1919** W. P. Lombard, President; C. W. Greene, Secretary; J. Erlanger, Treasurer; W. B. Cannon, Y. Henderson, J. J. R. Macleod, W. J. Meek, Councilors. **1920** W. P. Lombard, President; C. W. Greene, Secretary; J. Erlanger, Treasurer; W. B. Cannon, J. J. R. Macleod, Y. Henderson, C. J. Wiggers, Councilors. **1921** J. J. R. Macleod, President; C. W. Greene, Secretary; J. Erlanger, Treasurer; J. A. E. Eyster, Y. Henderson, C. J. Wiggers, A. J. Carlson, Councilors. **1922** J. J. R. Macleod, President; C. W. Greene, Secretary; J. Erlanger, Treasurer; Y. Henderson, C. J. Wiggers, A. J. Carlson, J. A. E. Eyster, Councilors. **1923** A. J. Carlson, President; C. W. Greene, Secretary; J. Erlanger, Treasurer; C. J. Wiggers, A. B. Luckhardt, J. A. E. Eyster, J. R. Murlin, Councilors. **1924** A. J. Carlson, President; W. J. Meek, Secretary; C. K. Drinker, Treasurer; A. B. Luckhardt, J. A. E. Eyster, J. R. Murlin, W. E. Garrey, Councilors. **1925** A. J. Carlson, President; W. J. Meek, Secretary; C. K. Drinker, Treasurer; J. A. E. Eyster, J. R. Murlin, W. E. Garrey, Joseph Erlanger, Councilors. **1926** J. Erlanger, President; W. J. Meek, Secretary; A. Forbes, Treasurer; J. R. Murlin, W. E. Garrey, A. B. Luckhardt, C. J. Wiggers, Councilors. **1927** J. Erlanger, President; W. J. Meek, Secretary; A. Forbes, Treasurer; W. E. Garrey, A. B. Luckhardt, C. J. Wiggers, R. Gesell, Councilors. **1928** J. Erlanger, President; W. J. Meek, Secretary; A. Forbes, Treasurer; A. B. Luckhardt, C. J. Wiggers, R. Gesell, A. J. Carlson, Councilors. **1929** W. J. Meek, President; Alfred C. Redfield, Secretary; A. Forbes, Treasurer; C. J. Wiggers, R. Gesell, A. J. Carlson, J. R. Murlin, Councilors. **1930** W. J. Meek, President; Arno B. Luckhardt, Secretary; A. Forbes, Treasurer; R. Gesell, A. J. Carlson, J. R. Murlin, E. G. Martin, Councilors. **1931** W. J. Meek, President; Arno B. Luckhardt, Secretary; Alexander Forbes, Treasurer; A. J. Carlson, J. R. Murlin, E. G. Martin, John Tait, Councilors. **1932** Arno B. Luckhardt, President; Frank C. Mann, Secretary; Alexander Forbes, Treasurer; E. G. Martin, W. J. Meek, J. R. Murlin, John Tait, Councilors. **1933** Arno B. Luckhardt, President; Frank C. Mann, Secretary; Alexander Forbes, Treasurer; Herbert S. Gasser, Ernest G. Martin, W. J. Meek, John Tait, Councilors. **1934** Charles W. Greene, President; Frank C. Mann, Secretary; Alexander Forbes, Treasurer; Herbert S. Gasser, Arno B. Luckhardt, W. J. Meek, John Tait, Councilors. **1935** Frank C. Mann, President; Andrew C. Ivy, Secretary; Alexander Forbes, Treasurer; Charles H. Best, Herbert S. Gasser, Arno B. Luckhardt, W. J. Meek, Councilors. **1936** Frank C. Mann, President; Andrew C. Ivy, Secretary; Wallace O. Fenn, Treasurer; Charles H. Best, Philip Bard, Herbert S. Gasser, Arno B. Luckhardt, Councilors. **1937** Walter E. Garrey, President; Andrew C. Ivy, Secretary; Wallace O. Fenn, Treasurer; Charles H. Best, Philip Bard, Herbert S. Gasser, Arno B. Luckhardt, Councilors. **1938** William T. Porter, Honorary President; Walter E. Garrey, President; Andrew C. Ivy, Secretary; Wallace O. Fenn, Treasurer; Arno B. Luckhardt, Charles H. Best, Philip Bard, Herbert S. Gasser, Councilors. **1939** Andrew C. Ivy, President; Philip Bard, Secretary; Wallace O. Fenn, Treasurer; Charles H. Best, Herbert S. Gasser, Arno B. Luckhardt, Maurice B. Visscher, Councilors. **1940** Andrew C. Ivy, President; Philip Bard, Secretary; Carl J. Wiggers, Treasurer; Charles H. Best, Herbert S. Gasser, Arno B. Luckhardt, Maurice B. Visscher, Councilors. **1941** Philip Bard, President; Carl J. Wiggers, Secretary; Hallowell Davis, Treasurer; Charles H. Best, Arno B. Luckhardt, Maurice B. Visscher, Hiram E. Essex, Councilors.

CONSTITUTION

I

1. This Society shall be named "The American Physiological Society."

2. The Society is instituted to promote the advance of Physiology and to facilitate personal intercourse between American Physiologists.

II

1. The Society shall consist of ordinary and of honorary members.

2. Any person who has conducted and published original researches in Physiology, and who is a resident of North America, shall be eligible for election as an ordinary member of the Society.

3. Distinguished men of science who have contributed to the advance of Physiology shall be eligible for election as honorary members of the Society. Honorary members shall pay no membership fee. They shall have the right of attending the meetings of the Society, and of taking part in its scientific discussions, but they shall have no vote.

III

1. The management of the Society shall be vested in a Council consisting of the President, Secretary, Treasurer, and four other members to be chosen from the ordinary members by ballot at each annual meeting. The President, Secretary, and Treasurer shall be elected for one year. The President shall be subject to only one reëlection. The four additional members of the Council shall be elected for a term of four years, and the term of office of one of these councilors shall expire at the close of each annual meeting. He or she shall not be eligible for reëlection for a period of two years.

2. The Council shall have power to fill such vacancies as may occur in its membership or in any committee of the Society unless the vacancy is produced by a resignation presented at a meeting.

IV

1. At least a fortnight before the annual meeting the Secretary shall send to each member a notice of the place and time of each meeting, and shall make such other announcements as the Council shall direct.

2. The annual assessment shall be determined by the Council, and shall be due in advance at the time of the annual meeting. Beyond the ordinary expenditures required by the duties of the Secretary and Treasurer, no money of the Society shall be disbursed save by authority of the Council.

3. Any member whose assessment is two years in arrears shall cease to be a member of the Society, unless at the next annual meeting he shall be reinstated by special vote of the Society; and it shall be the duty of the Treasurer to inform the Secretary that he may notify the said delinquent of his right to appeal to said meeting.

4. Any member who has paid the annual assessment for thirty years, or who has attained the age of sixty-five years, or who has retired because of illness, may be relieved from the payment of the annual assessment.

V

1. The annual meeting of the Society shall be held at a time and place determined by the Council in consultation with the Executive Committee of the Federation of American Societies for Experimental Biology.

2. Special meetings may be held at such times and places as the Council may determine.

VI

1. Proposed changes in the Constitution must be sent in writing to the Secretary at least one month before the date of the meeting at which they are to be considered, and must be signed by at least three ordinary members. The Secretary shall send a printed copy of any proposed change to each ordinary member at least two weeks before the next meeting.

2. If at this meeting two-thirds of the votes cast shall favor the proposed change, it shall be made.

3. At all annual meetings of the Society ten ordinary members shall form a quorum for the transaction of business.

VII

1. The Council may, from the names of the candidates proposed in writing by at least two ordinary members of the Society, nominate candidates for election to ordinary membership. The names of the candidates so nominated, together with the names of their proposers and a statement of their qualifications for membership, shall be posted on a bulletin board at an annual meeting of the Society. The candidates may be balloted for at any session of the same meeting, and one black ball in eight shall exclude.

2. Honorary members shall be proposed by the Council, and shall be elected by ballot of the members present at an annual meeting and one negative vote in twenty shall exclude.

VIII

If a majority of the Council shall decide that the interests of the Society require the expulsion of a member, the Secretary shall send a notice of this decision to each ordinary member at least two weeks before the next annual meeting. At this meeting the Secretary shall, on behalf of the Council, propose the expulsion; and if two-thirds of the members present vote for it, the member shall be expelled, his assessment for the current year shall be returned to him, and he shall cease to be a member of the Society.

IX

1. The offical organs of the Society shall be the *American Journal of Physiology* and such other Journals as the Society shall from time to time establish. These the Society shall own and manage.

2. The management of the *Journals* shall be vested in the Council. The Council shall make a full report to the Society at each annual meeting of the financial condition and the publication policy of the *Journals*.

BY-LAWS

1. All papers read before the Society shall be limited to a length of ten minutes. No person may orally present more than one paper. In case of joint authorship the name of the individual who will orally present the paper shall stand first.

2. Abstracts in duplicate, not to exceed two-hundred and fifty words in length, of all papers to be presented at the Annual Meeting of the Society shall be required by the Secretary for publication in the Federation Proceedings, in accordance with rules approved by the Council.

3. The Council may, upon the request of ten ordinary members, call a special meeting of the Society, at any time and place, for the reading of papers and the promotion of personal intercourse. Such meetings shall be held in accordance with the Constitution and By-Laws of the Society; and if the regular officers of the Society are not present, the members in attendance shall elect a temporary Chairman and Secretary. The latter officer shall forward an account of the scientific proceedings of the meeting to the offical Secretary of the Society for insertion in the minutes; he shall also prepare and transmit to the official Secretary such abstracts of papers read as may be furnished him, and these abstracts shall be published in the *Federation Proceedings* in accordance with By-Law 2.

THE AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, INCORPORATED

Founded December 6, 1906; Incorporated September 12, 1919

OFFICERS ELECTED 1942

President—R. J. Anderson, Sterling Chemistry Laboratory, Yale University, New Haven, Conn.

Vice-President—E. A. Doisy, St. Louis University School of Medicine, St. Louis, Mo.

Secretary—A. K. Balls, U. S. Bureau of Agricultural Chemistry and Engineering, Washington, D. C.

Treasurer—W. C. Stadie, Maloney Clinic, University of Pennsylvania, Philadelphia, Pa.

Councilors-at-large—W. C. Rose, University of Illinois, Urbana; C. G. King, Nutrition Foundation, Chrysler Building, New York City; H. T. Clarke, Columbia University, New York City.

Nominating Committee—V. du Vigneaud, Chairman, H. B. Vickery, C. A. Elvejhem, A. H. Smith, P. A. Shaffer, R. R. Williams, J. M. Luck, A. B. Hastings, D. D. Van Slyke.

PAST OFFICERS

1907 Russell H. Chittenden, President; J. J. Abel, Vice-President; W. J. Gies, Secretary; L. B. Mendel, Treasurer; W. Jones, W. Koch, J. Marshall, T. B. Osborne, Councilors. **1908** John J. Abel, President; Otto Folin, Vice-President; Wm. J. Gies, Secretary; L. B. Mendel, Treasurer; A. B. Macallum, A. P. Mathews, F. G. Novy, Councilors. **1909** Otto Folin, President; T. B. Osborne, Vice-President; Wm. J. Gies, Secretary; L. B. Mendel, Treasurer; J. J. Abel, P. A. Levene, G. Lusk, Councilors. **1910** Thomas B. Osborne, President; L. B. Mendel, Vice-President; A. N. Richards, Secretary; Walter Jones, Treasurer; A. B. Macallum, A. P. Mathews, V. C. Vaughan, Councilors. **1911** Lafayette B. Mendel, President; A. B. Macallum, Vice-President; A. N. Richards, Secretary; Walter Jones, Treasurer; Wm. J. Gies, A. S. Loevenhart, P. A. Shaffer, Councilors. **1912** Archibald B. Macallum, President; G. Lusk, Vice-President; A. N. Richards, Secretary; Walter Jones, Treasurer; H. P. Armsby, L. B. Mendel, H. G. Wells, Councilors. **1913** Archibald B. Macallum, President; G. Lusk, Vice-President; P. A. Shaffer, Secretary; D. D. Van Slyke, Treasurer; H. P. Armsby, L. B. Mendel, H. G. Wells, Councilors. **1914** Graham Lusk, President; C. L. Alsberg, Vice-President; P. A. Shaffer, Secretary; D. D. Van Slyke, Treasurer; J. J. Abel, A. B. Macallum, T. B. Osborne, Councilors. **1915** Walter Jones, President; C. L. Alsberg, Vice-President; P. A. Shaffer, Secretary; D. D. Van Slyke, Treasurer; Otto Folin, G. Lusk, L. B. Mendel, Councilors. **1916** Walter Jones, President; F. P. Underhill, Vice-President; S. R. Benedict, Secretary; D. D. Van Slyke, Treasurer; Otto Folin, A. B. Macallum, P. A. Shaffer, Councilors. **1917** Carl L. Alsberg, President; A. P. Mathews, Vice-President; S. R. Benedict, Secretary; H. C. Bradley, Treasurer; L. J. Henderson, P. A. Shaffer, F. P. Underhill, Councilors. **1918** Carl L. Alsberg, President; A. P. Mathews, Vice-President; S. R. Benedict, Secretary; H. C. Bradley, Treasurer; W. J. Gies, Andrew

HUNTER, E. V. MCCOLLUM, Councilors. **1919** STANLEY, R. BENEDICT, President; D. D. VAN SLYKE, Vice-President; V. C. MYERS, Secretary; H. C. BRADLEY, Treasurer; ANDREW HUNTER, E. V. MCCOLLUM, L. B. MENDEL, Councilors. **1920** STANLEY R. BENEDICT, President; D. D. VAN SLYKE, Vice-President; V. C. MYERS, Secretary; H. C. BRADLEY, Treasurer; OTTO FOLIN, WALTER JONES, L. B. MENDEL, Councilors. **1921** DONALD D. VAN SLYKE, President; P. A. SHAFFER, Vice-President; V. C. MYERS, Secretary; H. C. BRADLEY, Treasurer; S. R. BENEDICT, OTTO FOLIN, WALTER JONES, Councilors. **1922** DONALD D. VAN SLYKE, President; P. A. SHAFFER, Vice-President; V. C. MYERS, Secretary; W. R. BLOOR, Treasurer; S. R. BENEDICT, H. C. BRADLEY, A. P. MATHEWS, Councilors. **1923** PHILIP A. SHAFFER, President; H. C. SHERMAN, Vice-President; V. C. MYERS, Secretary; W. R. BLOOR, Treasurer; H. C. BRADLEY, ANDREW HUNTER, A. P. MATHEWS, Councilors. **1924** PHILIP A. SHAFFER, President; HENRY C. SHERMAN, Vice-President; D. WRIGHT WILSON, Secretary; WALTER R. BLOOR, Treasurer; OTTO FOLIN, ANDREW HUNTER, VICTOR C. MYERS, Councilors. **1925** HENRY C. SHERMAN, President; EDWARD C. KENDALL, Vice-President; D. WRIGHT WILSON, Secretary; WALTER R. BLOOR, Treasurer; OTTO FOLIN, LAFAYETTE B. MENDEL, PHILIP A. SHAFFER, Councilors. **1926** EDWARD C. KENDALL, President; ELMER V. MCCOLLUM, Vice-President; FRED C. KOCH, Secretary; GLENN E. CULLEN, Treasurer; J. B. COLLIP, EDWARD A. DOISY, ALBERT P. MATHEWS, Councilors. **1927** E. V. MCCOLLUM, President; W. R. BLOOR, Vice-President; D. WRIGHT WILSON, Secretary; G. E. CULLEN, Treasurer; E. A. DOISY, F. C. KOCH, D. D. VAN SLYKE, Councilors. **1928** E. V. MCCOLLUM, President; W. R. BLOOR, Vice-President; D. WRIGHT WILSON, Secretary; G. E. CULLEN, Treasurer; WM. M. CLARK, F. C. KOCH, D. D. VAN SLYKE, Councilors. **1929** W. R. BLOOR, President; H. C. BRADLEY, Vice-President; H. B. LEWIS, Secretary; G. E. CULLEN, Treasurer; W. M. CLARK, C. L. A. SCHMIDT, P. A. SHAFFER, Councilors. **1930** W. R. BLOOR, President; H. C. BRADLEY, Vice-President; H. B. LEWIS, Secretary; G. E. CULLEN, Treasurer; W. M. CLARK, P. A. SHAFFER, D. W. WILSON, Councilors. **1931** H. C. BRADLEY, President; W. M. CLARK, Vice-President; H. B. LEWIS, Secretary; C. H. FISKE, Treasurer; W. C. ROSE, P. A. SHAFFER, D. W. WILSON, Councilors. **1932** H. C. BRADLEY, President; W. M. CLARK, Vice-President; H. B. LEWIS, Secretary; C. H. FISKE, Treasurer; P. E. HOWE, W. C. ROSE, D. W. WILSON, Councilors. **1933** W. M. CLARK, President; H. B. LEWIS, Vice-President; H. A. MATTILL, Secretary; C. H. FISKE, Treasurer; H. C. BRADLEY, P. E. HOWE, W. C. ROSE, Councilors. **1934** W. M. CLARK, President; H. B. LEWIS, Vice-President; H. A. MATTILL, Secretary; C. H. FISKE, Treasurer; H. C. BRADLEY, E. A. DOISY, P. E. HOWE, Councilors. **1935** H. B. LEWIS, President; G. E. CULLEN, Vice-President; H. A. MATTILL, Secretary; C. H. FISKE, Treasurer; H. C. BRADLEY, J. B. COLLIP, E. A. DOISY, Councilors. **1936** H. B. LEWIS, President; G. E. CULLEN, Vice-President; H. A. MATTILL, Secretary; A. B. HASTINGS, Treasurer; J. B. COLLIP, E. A. DOISY, W. C. ROSE, Councilors. **1937** G. E. CULLEN, President; W. C. ROSE, Vice-President; H. A. MATTILL, Secretary; A. B. HASTINGS, Treasurer; E. A. DOISY, H. B. LEWIS, H. B. VICKERY, Councilors. **1938** G. E. CULLEN, President; W. C. ROSE, Vice-President; CHARLES G. KING, Secretary; A. B. HASTINGS, Treasurer; H. B. LEWIS, H. A. MATTILL, H. B. VICKERY, Councilors. **1939** W. C. ROSE, President; R. J. ANDERSON, Vice-President; CHARLES G. KING, Secretary; A. B. HASTINGS, Treasurer; H. B. LEWIS, H. A. MATTILL, G. E. CULLEN, Councilors. **1940** WILLIAM C. ROSE, President; RUDOLPH J. ANDERSON, Vice-President; CHARLES G. KING, Secretary; A. B. HASTINGS, Treasurer; H. A. MATTILL, GLENN E. CULLEN, E. A. DOISY, Councilors. **1941** R. J. ANDERSON, President; E. A. DOISY, Vice-President; A. K. BALLS, Secretary; W. C. STADIE, Treasurer; H. B. LEWIS, W. C. ROSE, Councilors.

CONSTITUTION

FROM THE ARTICLES OF INCORPORATION

1. The name of the proposed corporation is "AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, INCORPORATED."

2. The purposes for which this corporation is formed are to further the extension of biochemical knowledge and to facilitate personal intercourse between American investigators in biological chemistry.

BY-LAWS

ARTICLE I.—*Membership*

SECTION 1. *Eligibility for Membership.*—Qualified investigators who have conducted and published meritorious original investigations in biological chemistry shall be eligible for membership in the Society.

SEC. 2. *Nomination.*—Nominations for membership shall be made and seconded by members of the Society on blanks furnished by the Secretary. Nominations shall be submitted to the Council who shall determine eligibility and make recommendation to the Society at a regular meeting.

SEC. 3. *Election to Membership.*—A. A nominee for membership may be voted for by ballot at any meeting of the Society after Council has reported its findings on his eligibility. The eligible candi-

date shall be reported by the Council as "eligible" or as "eligible and indorsed." B. A majority of the ballots cast shall elect.

SEC. 4. *Forfeiture.*—A. Any member who may grant the use of his name for (a) the advertisement of a patent medicine, a proprietary food preparation, or any other commercial article of doubtful value to the public or possibly harmful to the public health, or (b) who may concede its use for the purpose of encouraging the sale of individual samples (of any such product) that he has not examined, shall forfeit his membership.

B. The Council shall have authority to announce forfeiture of membership, provided that the copy of the charges, together with a written notice of a hearing thereon by the Council at a place and time specified in such notice, shall have been delivered to the member charged with violating the preceding section either personally or mailed to him at his last known address at least thirty days before the date of such hearing.

SEC. 5. *Expulsion.*—Upon the recommendation of the Council any member may be expelled by a majority vote of the total membership at a meeting of the Society, provided that a copy of the charges against him, together with a written notice of a hearing thereon by the Council at a place and time specified in such notice shall have been delivered to him personally or mailed to him at his last known address at least thirty days before the date of such hearing.

ARTICLE II.—*Meetings and Quorum*

SECTION 1. *Annual.*—The annual meeting of the Society shall be held on the date fixed by the Certificate of Incorporation.

SEC. 2. *Special.*—A special meeting may be called at any time by the President, or in case of his absence or disability, by the Vice-President, and must be called at the request of a majority of the Council or fifteen members of the Society. A notice specifying the purpose of such meeting shall be mailed to each member at least ten days previous thereto. The Council shall select the places at which meetings shall be held.

SEC. 3. *Quorum.*—Fifteen members shall constitute a quorum at all meetings of the Society, but in absence of a quorum any number shall be sufficient to adjourn to a fixed date.

ARTICLE III.—*Officials*

SECTION 1. *Officers.*—The officers shall be a President, a Vice-President, a Secretary, and a Treasurer, who shall be elected annually by the members of the Society.

SEC. 2. *Council.*—A. The officers so elected and three additional members, one of whom shall be elected at each annual meeting of the Society to serve a three year term, shall constitute the Board of Directors of the corporation and shall be known as "The Council." (When this provision is first put into effect three members will need to be elected for a one, a two and a three year period.)

B. No two members of the Council may be from the same institution, and none of the officers so elected shall be eligible for re-election for more than two years except the Secretary and Treasurer, who shall be eligible for re-election for five years. The three additional members of the Council shall be ineligible for re-election (until after the lapse of one year).

SEC. 3. *Duties of Officers.*—The powers and duties of the officers elected by the Society shall be such as usually devolve upon their respective positions.

SEC. 4. *Assistant Treasurer.*—A. The Council may from time to time appoint a trust company, or some member of the Society, to serve during the pleasure of the Council as Assistant Treasurer, and to act as depositary of the investments and income of the "Christian A. Herter Memorial Fund" and of such other funds as the Society may from time to time commit to its or his charge.

B. The Assistant Treasurer shall have and exercise the following powers and duties, viz., the custody and safe-keeping of securities and cash belonging to the "Christian A. Herter Fund" and the collection of income and other moneys due to the Fund, with power to receipt for the same and to endorse for deposit all checks payable to the Society or the Treasurer, or to the Journal of Biological Chemistry for income or other moneys due to the Fund, the investment or reinvestment of the capital of the Fund, subject to the approval of the Council; the disbursement of principal under the direction of the Council and the disbursement of income under the direction of the Editorial Board of the Journal of Biological Chemistry, such disbursement to be made under a resolution of the Council or Board, or with the approval of two members of either the Council or Board, as the case may be. The Assistant Treasurer shall keep books of account and render statments, annually or oftener upon the request of the Council or Board setting forth the condition of the Fund and the recepits and disbursements since the date of the preceding statement.

ARTICLE IV.—*The Council*

SECTION 1. *Powers.*—The general management of the Society during the intervals between meetings shall be vested in the Council, which shall regularly perform the ordinary duties of an executive committee and possess all the powers conferred upon the Board of Directors of a membership corporation by the Membership Corporation Law of the State of New York.

SEC. 2. *Reports.*—The Council shall report to the Society as promptly as possible its findings on the eligibility of candidates for membership, and on all charges of a violation of these By-Laws.

SEC. 3. *Journal of Biological Chemistry.*—The Council shall have power to appoint the persons to act as proxies for the Society at all meetings of the stockholders of the "Journal of Biological Chemistry" (a corporation) of which all the stock is owned by the Society, and also to designate the persons to be elected as Directors of such corporation.

SEC. 4. *Herter Fund.*—It shall be the duty of the Council to see that the "Christian A. Herter Memorial Fund" is administered in accordance with the terms of the Trust Agreement, dated May 16, 1911, executed by the Journal of Biological Chemistry and the donors of said Fund.

ARTICLE V.—*Nominating Committee*

SECTION 1. *Membership.*—A. The Nominating Committee shall consist of nine members from nine different institutions elected at each annual meeting to serve for the ensuing year. Members who have served on the Nominating Committee for two consecutive years shall be ineligible for re-election until after the lapse of one year.

B. The member of the Nominating Committee who is elected to the Committee by the largest number of votes shall become Chairman and Secretary of the Committee.

SEC. 2. *Nomination of Officials.*—A. The Nominating Committee shall make at least one nomination for each of the four offices and for each of the three additional positions in the Council to be filled by vote of the members.

B. The nominations by the Nominating Committee must be transmitted to the Secretary at least one month before the annual meeting at which they are to be considered.

C. The Secretary shall send to every member, at least two weeks before the annual meeting, two copies of the list of nominees presented to him by the Nominating Committee and at the same time shall notify all the members that they may vote by proxy.

D. At the opening of the first executive session of the ensuing annual meeting the Secretary shall formally present the regular nominations for the Nominating Committee.

E. Additional nominations for the offices and for membership in the Council may be made by any member at the opening of the first executive session of any annual meeting.

F. Nominations for membership on the Nominating Committee shall be made by or for individual members, either in person or by proxy, and not otherwise, at the opening of the first executive session of any annual meeting.

SEC. 3. *Election of Officials.*—A. The Secretary shall receive and present to the tellers, appointed by the President to take charge of the election, all signed ballots forwarded by absent members. When such ballots are presented to the tellers the Secretary shall announce the names of the members voting by proxy, and he shall record the same names in the minutes of the meeting.

B. All elective officials shall be selected by ballot at the close of the first executive session of each annual meeting.

C. A majority of the votes cast shall be necessary to elect an official.

D. Elective officials shall take office on July 1st following the annual meeting.

SEC. 4. *Filling of Vacancies.*—A. The Nominating Committee shall fill all vacancies in elective positions except such as may occur at a meeting of the Society.

B. The President of the Society shall fill all vacancies in appointive positions.

ARTICLE VI.—*Financial*

SECTION 1. *Dues.*—Annual assessments shall be determined by majority vote at the annual meetings, upon the recommendation of the Council, and shall be due January 15th in each year. Members who have reached the age of 65 years, or who have become incapacitated, may, by vote of the Council, be exempted from the payment of dues.

SEC. 2. *Expenditures.*—No expenditures from the general funds of the Society except those required in the performance of the ordinary official duties shall be made except by vote of the Society or the Council, but this section shall not apply to expenditures from the "Christian A. Herter Memorial Fund."

SEC. 3. *Privileges of Membership Begin with Payment of Dues.*—Candidates for membership, if elected, shall not be entitled to any of the privileges of membership, before they pay the dues of the fiscal year succeeding their election.

SEC. 4. *Penalty for Non-Payment of Dues.*—A. Members in arrears for dues for a period of three consecutive years shall thereupon forfeit their membership.

B. Delinquent members may be reinstated by the Council provided all indebtedness to the Society is liquidated.

SEC. 5. *Herter Fund.*—The "Christian A. Herter Memorial Fund" shall be held and invested separately from the general funds of the Society and the income thereof shall be expended under the direction of the Editorial Board exclusively for the maintenance and support of the Journal of Biological Chemistry, subject to the supervision and control of the Editorial Committee in accordance with the terms of the Trust Agreement

mentioned in ARTICLE IV, SECTION 4, and the provisions of ARTICLE VII of the By-Laws.

ARTICLE VII.—*Journal of Biological Chemistry*

SECTION 1. *Editorial Committee.*—There shall be an Editorial Committee consisting of nine members of the Society who shall be nominated by the Nominating Committee and elected by the Society in the same manner as officers. The nine members first elected shall divide themselves by lot into three classes of three in each class, to serve for two, four, and six years respectively, and thereafter three members shall be elected at each alternate annual meeting of the Society to succeed the members of the outgoing class and to serve for a term of six years. Members of the Committee shall be eligible to re-election.

SEC. 2. *Powers of Committee.*—The Committee shall have power to elect an Editorial Board and shall have final authority in matters pertaining to the general policy of the Journal.

SEC. 3. *Editorial Board.*—The members of the Board shall hold office until their successors are elected and shall appoint a Managing Editor from among their own number who shall have direct responsibility and authority for the active editorial conduct of the Journal, and who shall have discretionary power in arranging the details as to the conduct of the Journal. The expenditures of the income of the "Christian A. Herter Memorial Fund" shall be under the direction of the Board, and the approval of any two members of the Board shall be a sufficient warrant to authorize payments from such income.

ARTICLE VIII.—*Papers on Scientific Subjects*

SECTION 1. *Presentation of Papers.*—The Secretary shall request each member who signifies his intention of reading a paper at any session to specify the length of time which its presentation will require. The time thus specified shall be printed on the official program, and the presiding officer shall have no authority to extend it unless a majority of the members present signify their wish to the contrary. In the absence of any specification of time required not more than ten minutes shall be allotted for the reading of any one paper.

SEC. 2. *Number of Papers.*—No member shall be permitted to present more than one paper, either alone or in collaboration, until every member shall have had the opportunity of presenting one paper.

ARTICLE IX.—*Corporate Seal*

SECTION 1. The corporate seal of the corporation shall be a circle surrounded by the words, "AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS," and including the word, "INCORPORATED."

ARTICLE X.—*Amendments*

SECTION 1. *Amendments.*—These By-Laws, after having been approved by the Council, and adopted by the Society at its first annual meeting, shall not be amended except as hereinafter provided.

SEC. 2. *Manner of Presentation.*—Proposed amendments to the By-Laws must be sent to the Secretary at least one month before the date of the meeting at which they are to be considered and must be indorsed in writing by at least three members.

SEC. 3. *Notice of Intended Amendments.*—The Secretary shall give every member notice of proposed amendments at least two weeks before the meeting at which they are to be considered and shall notify all members that they may vote by proxy.

SEC. 4. *Adoption of Amendments.*—A. The Secretary shall receive and present to the tellers appointed by the President all signed ballots forwarded by absent members. When such ballots are presented to the tellers, the Secretary shall announce the names of members voting by proxy, and he shall record the same names in the minutes of the meeting.

B. Votes upon amendments shall be cast at the opening of the second executive session of the meeting at which they are considered.

C. Affirmative votes from three-fifths of the members voting shall be required for the adoption of an amendment.

AMERICAN SOCIETY FOR PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS, INCORPORATED

Founded December 28, 1908; Incorporated June 19, 1933

OFFICERS ELECTED 1942

President—E. K. MARSHALL, JR., The Johns Hopkins School of Medicine, Baltimore, Maryland.

Vice-President—CARL A. DRAGSTEDT, Northwestern University Medical School, Chicago, Illinois.

Secretary—RAYMOND N. BIETER, University of Minnesota Medical School, Minneapolis.

Treasurer—E. E. NELSON, Tulane University of Louisiana, Station 20, New Orleans, Louisiana.

Council—McK. CATTELL, Cornell University Medical College, New York, New York, R. G. SMITH, University of Michigan, Ann Arbor, E. K. MARSHALL, JR., C. A. DRAGSTEDT, R. N. BIETER, E. E. NELSON.

Membership Committee—HARRY GOLD (term expires 1943), Cornell University Medical College, New York, New York, M. H. SEEVERS (term expires 1944), University of Michigan, Ann Arbor, Michigan, HARVEY B. HAAG (term expires 1945), Medical College of Virginia, Richmond, Virginia.

Nominating Committee—H. O. CALVERY, Chairman, A. C. DEGRAFF, J. M. DILLE, B. H. ROBBINS, F. F. YONKMAN.

PAST OFFICERS

1909 J. J. ABEL, President; REID HUNT, Secretary; A. S. LOEVENHART, Treasurer; S. J. MELTZER, T. SOLLMANN, C. W. EDMUNDS, A. C. CRAWFORD, Councilors. **1910** J. J. ABEL, President; REID HUNT, Secretary; A. S. LOEVENHART, Treasurer; A. C. CRAWFORD, G. B. WALLACE, Councilors. **1911** J. J. ABEL, President; REID HUNT, Secretary; A. S. LOEVENHART, Treasurer; G. B. WALLACE, W. DEB. MACNIDER, Councilors. **1912** J. J. ABEL, President; J. AUER, Secretary; A. S. LOEVENHART, Treasurer; G. B. WALLACE, REID HUNT, Councilors. **1913** T. SOLLMANN, President; J. AUER, Secretary; A. S. LOEVENHART, Treasurer; J. J. ABEL, W. DEB. MACNIDER, Councilors. **1914** T. SOLLMANN, President, J. AUER, Secretary; W. DEB. MACNIDER, Treasurer; J. J. ABEL, A. S. LOEVENHART, Councilors. **1915** T. SOLLMANN, President; J. AUER, Secretary; W. DEB. MACNIDER, Treasurer; WORTH HALE, D. E. JACKSON, Councilors. **1916** REID HUNT, President; J. AUER, Secretary; W. DEB. MACNIDER, Treasurer; A. D. HIRSCHFELDER, G. B. ROTH, Councilors. **1917** REID HUNT, President; L. G. ROWNTREE, Secretary; W. DEB. MACNIDER, Treasurer; J. AUER, CARL VOEGTLIN, Councilors. **1918** REID HUNT, President; E. D. BROWN, Secretary; W. DEB. MACNIDER, Treasurer; HUGH MCGUIGAN, CARL VOEGTLIN, Councilors. **1919** A. S. LOEVENHART, President; E. D. BROWN, Secretary; W. DEB. MACNIDER, Treasurer; REID HUNT, E. K. MARSHALL, JR., Councilors. **1920** A. S. LOEVENHART, President; E. D. BROWN, Secretary; W. DEB. MACNIDER, Treasurer; D. E. JACKSON, E. K. MARSHALL, JR., Councilors. **1921** C. W. EDMUNDS, President; E. D. BROWN, Secretary; HUGH MCGUIGAN, Treasurer; JOHN AUER, J. P. HANZLIK, Councilors. **1922** C. W. EDMUNDS, President; E. D. BROWN, Secretary; HUGH MCGUIGAN, Treasurer; J. P. HANZLIK, H. G. BARBOUR, Councilors. **1923** C. W. EDMUNDS, President; E. D. BROWN, Secretary; HUGH MCGUIGAN, Treasurer; J. P. HANZLIK, H. G. BARBOUR, Councilors. **1924** JOHN AUER, President; E. D. BROWN, Secretary; A. L. TATUM, Treasurer; J. P. HANZLIK, H. G. BARBOUR, Councilors. **1925** JOHN AUER, President; E. D. BROWN, Secretary; A. L. TATUM, Treasurer; H. G. BARBOUR, W. DEB. MACNIDER, Councilors. **1926** JOHN AUER, President; E. D. BROWN, Secretary; A. L. TATUM, Treasurer; H. G. BARBOUR, W. DEB. MACNIDER, Councilors. **1927** CARL VOEGTLIN, President; E. D. BROWN, Secretary; A. L. TATUM, Treasurer; V. E. HENDERSON, C. W. EDMUNDS, Councilors. **1928** CARL VOEGTLIN, President; E. D. BROWN, Secretary; A. L. TATUM, Treasurer; V. E. HENDERSON, C. W. EDMUNDS, Councilors. **1929** CARL VOEGTLIN, President; E. D. BROWN, Secretary; O. H. PLANT, Treasurer; V. E. HENDERSON, C. W. EDMUNDS, Councilors. **1930** GEORGE B. WALLACE, President; E. D. BROWN, Secretary; O. H. PLANT, Treasurer; H. G. BARBOUR, C. M. GRUBER, Councilors. **1931** GEORGE B. WALLACE, President; VELYIEN E. HENDERSON, Secretary; O. H. PLANT, Treasurer; PAUL D. LAMSON, WILLIAM DEB. MACNIDER, Councilors. **1932** WM. DEB. MACNIDER, President; A. N. RICHARDS, Vice-President; V. E. HENDERSON, Secretary; O. H. PLANT, Treasurer; G. B. ROTH, A. L. TATUM, Councilors. **1933** WM. DEB. MACNIDER, President; A. L. TATUM, Vice-President; V. E. HENDERSON, Secretary; O. H. PLANT, Treasurer; C. M. GRUBER, G. B. ROTH, Councilors. **1934** R. A. HATCHER, President; A. L. TATUM, Vice-President; E. M. K. GEILING, Secretary; O. H. PLANT, Treasurer; WM. DEB. MACNIDER, R. L. STEHLE, Councilors. **1935** V. E. HENDERSON, President; O. H. PLANT, Vice-President; E. M. K. GEILING, Secretary; C. M. GRUBER, Treasurer; FLOYD DE EDS, M. S. DOOLEY, Councilors. **1936** V. E. HENDERSON, President; O. H. PLANT, Vice-President; E. M. K. GEILING, Secretary; C. M. GRUBER, Treasurer; C. W. EDMUNDS, G. B. WALLACE, Councilors. **1937** A. L. TATUM, President; E. M. K. GEILING, Vice-President; G. P. GRABFIELD, Secretary; C.

M. GRUBER, Treasurer; V. E. HENDERSON, M. H. SEEVERS, Councilors. **1938** A. L. TATUM, President; E. M. K. GEILING, Vice-President; G. P. GRABFIELD, Secretary; C. M. GRUBER, Treasurer; E. K. MARSHALL, JR., C. F. SCHMIDT, Councilors. **1939** O. H. PLANT, President; E. M. K. GEILING, Vice-President; G. P. GRABFIELD, Secretary; E. E. NELSON, Treasurer; A. L. TATUM, C. A. DRAGSTEDT, Councilors. **1940** E. M. K. GEILING, President; C. F. SCHMIDT, Vice-President; G. PHILIP GRABFIELD, Secretary; E. E. NELSON, Treasurer; B. H. ROBBINS, C. H. THIENES, Councilors. **1941** E. M. K. GEILING, President; C. F. SCHMIDT, Vice-President; RAYMOND N. BIETER, Secretary; E. E. NELSON, Treasurer; E. G. GROSS, R. G. SMITH, Councilors.

CONSTITUTION

ARTICLE I.—*Name*

The name of this organization shall be the "AMERICAN SOCIETY FOR PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS, INCORPORATED."

ARTICLE II.—*Objects*

The purpose of this Society shall be to promote these branches of science and to facilitate personal intercourse between investigators who are actively engaged in research in these fields.

ARTICLE III.—*Membership*

SECTION 1. Any person who has conducted and published a meritorious investigation in pharmacology or experimental therapeutics, and who is an active investigator in one of these fields, shall be eligible to membership, subject to the conditions of the other sections of Article III.

SEC. 2. A. Candidates for membership to this Society shall be proposed by two members who are not members of the Council. The names so proposed shall be sent to the Secretary at least three months prior to the Annual Meeting.

B. The Membership Committee shall investigate the qualifications of the candidates and report to the Council.

C. Candidates reported upon by the Membership Committee to the Council may be recommended for admission by the Council only provided they have been unanimously approved by both the Membership Committee and the Council.

D. The names of the candidates recommended for admission by the Council shall be posted by the Secretary not later than the day preceding the election for members.

E. The election of members shall be by individual ballot; one opposing vote in every eight cast shall be sufficient to exclude a candidate from membership.

SEC. 3. *Forfeiture of Membership.*

A. Any member whose assessment is three years in arrears shall cease to be a member of the Society, unless he shall be reinstated by a special vote of the Council; and it shall be the duty of the Treasurer to inform the Secretary that he may notify the said delinquent of his right to appeal to the Council.

B. If the Council shall decide that it is for the best interests of the Society that a member be expelled, the member shall be notified and given an opportunity of a hearing before the Council. Upon the recommendation of the Council the member then may be expelled by a three-fourths vote of those present at a regular meeting of the Society.

SEC. 4. *Honorary Members.*

A. Distinguished men of science who have contributed to the advance of pharmacology or experimental therapeutics shall be eligible for election as honorary members of the Society.

B. Nominations for honorary members shall take the same course as nominations for ordinary members (Art. III, Sec. 2); but their election shall require the unanimous vote of the members present at the election.

C. Honorary members shall pay no membership fee. They shall have the right to attend all meetings of the Society, and to take part in its discussions, but they shall have no vote.

D. The conditions for continuation of membership shall be the same for honorary as for ordinary members (Art. III, Sec. 3), except that forfeiture for arrears of fees does not apply to honorary members.

ARTICLE IV.—*Officers and Elections*

SECTION 1. The management of the Society shall be vested in a Council of six officers, consisting of a President, a Vice President, a Secretary, a Treasurer, and two additional members.

SEC. 2. There shall be a Membership Committee consisting of three members, and a Nominating Committee consisting of five members. No two members of either Committee shall be from the same institution.

SEC. 3. Members of the Council shall serve for one year but they shall be eligible for re-election.

SEC. 4. The election of the Membership Committee shall be held annually at the time when the election of officers occurs. At the first meeting of the Society under this Constitution, one member shall be elected to serve on the Committee for three years, one for two years, and one for one year; and subsequently one member shall be elected each year to serve for a period of three years.

SEC. 5. A. Members of the Nominating Committee shall serve for one year. They are eligible for re-election, but shall not hold membership in the Committee for more than two consecutive years.

B. The Nominating Committee shall make at least one nomination for each office and for position on the Membership Committee to be filled by vote of the members. The nominations so made shall be transmitted to the Secretary and by him in turn to the members, at least one month before the annual meeting. Additional nominations may be made by any member at the time of the annual meeting.

C. Nominations for membership on the Nominating Committee shall be made by individual members at the time of the annual election. The five nominees who receive the highest number of votes shall be declared elected. The Nominating Committee shall select its own chairman who shall also serve as secretary to the Committee.

SEC. 6. The election of officers shall be held at the close of the first session of the annual meeting. In voting there shall be a ballot in regular order for each office to be filled, and the majority of the votes cast shall be necessary to a choice.

SEC. 7. Such vacancies as may occur in the offices and in the various committees in the interval between annual meetings shall be filled by a majority vote of the Council.

ARTICLE V.—*Meetings*

SECTION 1. The annual meeting of the Society shall be held at a time and place determined by the Council in consultation with the Executive Committee of the Federation of American Societies for Experimental Biology.

SEC. 2. Special meetings may be held at such times and places as the Council may determine.

SEC. 3. At least four weeks before the annual meeting the Secretary shall send to each member a notice of the time and place of such meeting and shall make such announcements as the Council may direct.

ARTICLE VI.—*Financial*

SECTION 1. The annual assessment shall be determined by majority vote at the annual meetings, upon the recommendation of the Council, and shall be due in advance at the time of the meeting.

SEC. 2. Beyond the ordinary expenditures required by the routine business of the Society no money shall be disbursed save by the authority of the Council or Society.

SEC. 3. The treasurer shall make an annual report to the Society.

SEC. 4. In case any profits result to the Society from the Journal of Pharmacology and Experimental Therapeutics at the end of the financial year, such profits shall be kept in a special account, after deducting any sums expended by the Society during the year for the conduct of the Journal, and shall be held subject to the order of the Council on recommendation of the Editorial Board.

ARTICLE VII.—*Quorum*

Ten members shall constitute a quorum for the transaction of business.

ARTICLE VIII.—*By-Laws*

By-Laws shall be adopted, altered or repealed at any meeting by two-thirds vote of the ballots cast.

ARTICLE IX.—*Amendments*

SECTION 1. Intended amendments to the Constitution shall be sent to the Secretary at least one month before the date of the meeting at which they are to be considered, and must be indorsed in writing by at least three members.

SEC. 2. The Secretary shall give all members due notice of proposed amendments.

SEC. 3. A four-fifths vote of the members present shall be required for the adoption of an amendment.

ARTICLE X.—*Journal*

SECTION 1. The official publication of the Society shall be the Journal of Pharmacology and Experimental Therapeutics.

SEC. 2. The Society shall elect an Editor-in-Chief for a term of three years and he with the approval of the Council shall appoint an Editorial Board of six members for a term of three years.

SEC. 3. The Editorial Board shall have direct authority and responsibility for the active editorial conduct of the Journal of Pharmacology and Experimental Therapeutics and shall have discretionary power in arranging details as to the conduct of the Journal.

BY-LAWS

1. Papers to be read shall be selected by the President and Secretary, who shall be empowered to arrange the program in their discretion. Papers not read shall appear on the program as read by title. No member shall be permitted to read or have read by title more than one paper.

2. An abstract of a paper to be read before the Society shall be sent to the Secretary with the title. As early as possible after each meeting, the Secretary shall edit and publish the Proceedings of the Society together with abstracts in a publication authorized by the Society.

3. All applications for membership shall be accompanied by a copy of as many reprints as possible of the published work of the applicant.

THE AMERICAN SOCIETY FOR EXPERIMENTAL PATHOLOGY

Founded December 29, 1913

OFFICERS ELECTED 1942

President—BALDUIN LUCKÉ, University of Pennsylvania Medical School, Philadelphia.

Vice-President—PAUL R. CANNON, University of Chicago, Chicago, Illinois.

Secretary-Treasurer—H. P. SMITH, College of Medicine, State University of Iowa, Iowa City.

Councilors—DOUGLAS H. SPRUNT, Duke University School of Medicine, Durham, N. C., FRIEDA S. ROBSCHEIT-ROBBINS, University of Rochester School of Medicine and Dentistry, Rochester, N. Y.

PAST OFFICERS

1914 R. M. PEARCE, President; JOHN F. ANDERSON, Vice-President; G. H. WHIPPLE, Secretary-Treasurer; HARVEY CUSHING, DAVID MARINE, Councilors. **1915** THEOBALD SMITH, President; G. H. WHIPPLE, Vice-President; PEYTON ROUS, Secretary-Treasurer; DAVID MARINE, R. M. PEARCE, Councilors. **1916** SIMON FLEXNER, President; LEO LOEB, Vice-President; PEYTON ROUS, Secretary-Treasurer; DAVID MARINE, R. M. PEARCE, Councilors. **1917** LUDVIG HEKTOEN, President; LEO LOEB, Vice-President; HOWARD T. KARSNER, Secretary-Treasurer; PAUL A. LEWIS, L. G. ROWNTREE, Councilors. **1918** H. GIDEON WELLS, President; W. G. MACCALLUM, Vice-President; HOWARD T. KARSNER, Secretary-Treasurer; L. G. ROWNTREE, LUDVIG HEKTOEN, Councilors. **1919** W. G. MACCALLUM, President; WILLIAM H. PARK, Vice-President; HOWARD T. KARSNER, Secretary-Treasurer; LUDVIG HEKTOEN, E. L. OPIE, Councilors. **1920** WILLIAM H. PARK, President; F. G. NOVY, Vice-President; HOWARD T. KARSNER, Secretary-Treasurer; E. L. OPIE, WADE H. BROWN, Councilors. **1921** F. G. NOVY, President; HOWARD T. KARSNER, Vice-President; WADE H. BROWN, Secretary-Treasurer; PAUL A. LEWIS, A. R. DOCHEZ, Councilors. **1922** HOWARD T. KARSNER, President EUGENE L. OPIE, Vice-President; WADE H. BROWN, Secretary-Treasurer; A. R. DOCHEZ, GEORGE H. WHIPPLE, Councilors. **1923** EUGENE L. OPIE, President; ALDRED S. WARTHIN, Vice-President; WADE H. BROWN, Secretary-Treasurer; GEORGE H. WHIPPLE, H. GIDEON WELLS, Councilors. **1924** ALDRED S. WARTHIN, President; GEORGE H. WHIPPLE, Vice-President; EDWARD B. KRUMBHAAR, Secretary-Treasurer; H. GIDEON WELLS, FREDERICK L. GATES, Councilors. **1925** GEORGE H. WHIPPLE, President; WADE H. BROWN, Vice-President; EDWARD B. KRUMBHAAR, Secretary-Treasurer; FREDERICK L. GATES, DAVID MARINE, Councilors. **1926** WADE H. BROWN, President; DAVID MARINE, Vice-President; EDWARD B. KRUMBHAAR, Secretary-Treasurer; FREDERICK L. GATES, WILLIAM F. PETERSEN, Councilors. **1927** DAVID MARINE, President; EDWARD B. KRUMBHAAR, Vice-President; CARL V. WELLER, Secretary-Treasurer; WILLIAM F. PETERSEN, FREDERICK L. GATES, Councilors. **1928** EDWARD B. KRUMBHAAR, President; WILLIAM F. PETERSEN, Vice-President; CARL V. WELLER, Secretary-Treasurer; FREDERICK L. GATES, SAMUEL R. HAYTHORN, Councilors. **1929** WILLIAM F. PETERSEN, President; FREDERICK L. GATES, Vice-President; CARL V. WELLER, Secretary-Treasurer; SAMUEL R. HAYTHORN, PEYTON ROUS, Councilors. **1930** FREDERICK L. GATES, President; SAMUEL R. HAYTHORN, Vice-President; C. PHILLIP MILLER, Secretary-Treasurer; PEYTON ROUS, CARL V. WELLER, Councilors. **1931** SAMUEL R. HAYTHORN, President; PEYTON ROUS, Vice-President; C. PHILLIP MILLER, Secretary-Treasurer; CARL V. WELLER, S. BURT WOLBACH, Councilors. **1932** PEYTON ROUS, President; CARL V. WELLER, Vice-President; C. PHILLIP MILLER, Secretary-Treasurer; S. BURT WOLBACH, OSKAR KLOTZ, Councilors. **1933** CARL V. WELLER, President; S. BURT WOLBACH, Vice-President; C. PHILLIP MILLER, Secretary-Treasurer; OSKAR KLOTZ, ALPHONSE R. DOCHEZ, Councilors. **1934** S. BURT WOLBACH, President; OSCAR KLOTZ, Vice-President; SHIELDS WARREN, Secretary-Treasurer; C. PHILLIP MILLER, ALPHONSE R. DOCHEZ, Councilors. **1935** OSKAR KLOTZ, President; ALPHONSE R. DOCHEZ, Vice President. SHIELDS WARREN, Secretary-Treasurer; MORTON MCCUTCHEON, C. PHILLIP MILLER, Councilors. **1936** ALPHONSE R. DOCHEZ, President; C. PHILLIP MILLER, Vice-President; SHIELDS WARREN, Secretary-Treasurer; MORTON MCCUTCHEON, ERNEST W. GOODPASTURE, Councilors. **1937** C. PHILLIP MILLER, President; MORTON MCCUTCHEON, Vice-President; PAUL R. CANNON, Secretary-Treasurer; ERNEST W. GOODPASTURE, SHIELDS WARREN, Councilors. **1938** MORTON MCCUTCHEON, President; ERNEST W. GOODPASTURE, Vice President; PAUL R. CANNON, Secretary-Treasurer; SHIELDS WARREN, JESSE L. BOLLMAN, Councilors. **1939** ERNEST W. GOODPASTURE, President; SHIELDS WARREN, Vice-President; PAUL R. CANNON, Secretary-Treasurer; JESSE L BOLLMAN, BALDUIN LUCKÉ, Councilors. **1940** SHIELDS WARREN President; JESSE L. BOLLMAN, Vice-President: H. P. SMITH, Secretary-Treasurer; BALDUIN LUCKÉ, PAUL R. CANNON, Councilors. **1941** JESSE L. BOLLMAN, President; BALDUIN LUCKÉ, Vice-President; H P. SMITH,

Secretary-Treasurer; PAUL R. CANNON, DOUGLAS H. SPRUNT, Councilors

CONSTITUTION

ARTICLE I.—*Name*

The Society shall be named "THE AMERICAN SOCIETY FOR EXPERIMENTAL PATHOLOGY."

ARTICLE II.—*Object*

The object of this Society is to bring the productive investigators in pathology, working essentially by experimental methods, in closer affiliation with the workers in the other fields of experimental medicine.

ARTICLE III.—*Time and Place of Meeting*

The Society shall meet at the same time and place as the Federation of American Societies for Experimental Biology, which comprises at present the American Physiological Society, the American Society of Biological Chemists, the American Society for Pharmacology and Experimental Therapeutics, and the American Society for Experimental Pathology.

ARTICLE IV.—*Membership*

SECTION 1. Any American investigator who, through the use of experimental methods, has, within three years prior to his candidacy, contributed meritorious work in pathology, is eligible to membership.

SEC. 2. It shall be the policy of the Society to restrict its membership to as small numbers as is compatible with the maintenance of an active existence.

SEC. 3. There shall be two classes of members: active and honorary members.

Active members: Candidates for active membership shall be nominated at or before an annual meeting by two members of the Society. The nominators shall present to the Secretary in writing evidence of the candidate's qualifications for membership. Nominations approved by the Council shall be presented to the Society for election at the next annual meeting following nomination. For election a favorable ballot by a majority of the members present is necessary.

Honorary members: These may be elected from the active list or from the group of distinguished investigators at home or abroad who have contributed to the knowledge of pathology by experimental study. They shall be elected only by the unanimous vote of the members present at time of nomination.

SEC. 4. Active members shall pay such annual dues as are determined upon, from year to year, by the Council. Honorary members shall pay no dues, are not eligible to office, and have no vote in the business affairs of the Society, but they shall have all the privileges of the active members in the scientific proceedings.

SEC. 5. Upon failure of an active member to pay dues for two years, notice shall be given to the member by the Secretary. At the end of the third year, if dues are still unpaid, such failure constitutes forfeiture of membership.

SEC. 6. A motion for expulsion of a member must be thoroughly investigated by the Council; at this investigation the accused shall be afforded a hearing or may be represented by a member. Expulsion can be accomplished only after a unanimous vote by the Council in favor of expulsion, sustained by a four-fifths vote of the members present at the meeting.

ARTICLE V.—*Officers*

The management of the Society shall be vested in a Council of five members, consisting of a President, a Vice-President, a Secretary-Treasurer, and two other members who shall be nominated by the Council and elected by the Society. Officers are elected by a majority vote. Vacancies shall be filled by the Council for the unexpired term.

The President and Vice-President shall hold office for one year and are ineligible for re-election during the following year. The Secretary-Treasurer is eligible for re-election. Councilors shall hold office for two years and are elected on alternate years. At the first election one Councilor shall be elected for a short term of one year.

ARTICLE VI.—*Quorum*

SECTION 1. Three constitute a quorum of the Council. The Council decides by a majority vote.

SEC. 2. A quorum of the Society for transaction of business shall be one-fourth of the total membership. In all questions brought before the Society a majority vote of those present shall decide, except as elsewhere provided for.

ARTICLE VII.—*Annual Meeting*

SECTION 1. Papers shall be limited to ten minutes. However, on motion and with unanimous consent, the time may be prolonged by a period not exceeding five minutes. The Council may make provision for longer papers on suitable occasions.

SEC. 2. The subjects of papers must be confined to experimental work in pathology. In doubtful cases a liberal interpretation by the President and Secretary may prevail. The Council may invite, however, presentations dealing with any subject which it considers of considerable interest to the Society.

ARTICLE VIII.—*Change of Constitution*

A motion concerning a change of the Constitution must be presented to the Council in writing by three members, and must be communicated to the members by the Secretary at least four weeks before the annual meeting. At this meeting such a change may be established when accepted by a four-fifths vote of the members present.

BY-LAWS

1. There must be in each year at least one meeting of the Council, which shall take place not later than the evening before the annual meeting.

2. At the end of the first session of the annual meeting the Secretary shall read the report of the Council. This report shall include (1) names of persons recommended for membership, (2) nominations for offices, (3) matters of general interest. The Secretary shall exhibit in a conspicuous place the names of candidates for membership recommended by the Council, together with the evidence of the qualifications of the candidates.

3. The election of officers and of new members, changes in the Constitution, etc., shall be voted upon at the end of the first session.

4. Changes in the By-Laws may be determined by a majority vote of those present.

5. In the year that a new Secretary-Treasurer is elected the incoming Council Member elected that year, or another member of the Council, shall become Assistant Secretary-Treasurer for the duration of the term of the Secretary-Treasurer.

THE AMERICAN INSTITUTE OF NUTRITION

Founded April 11, 1933; Incorporated November 16, 1934
Member of Federation 1940

OFFICERS ELECTED 1942

President—L. A. MAYNARD.
Vice-President—H. B. LEWIS.
Secretary—ARTHUR H. SMITH.
Treasurer—W. H. SEBRELL, JR.
Councilors—LYDIA J. ROBERTS, GENEVIEVE STEARNS and T. H. JUKES.
Nominating Committee—W. H. CHAMBERS, Chairman, H. J. ALMQUIST, N. B. GUERRANT, C. G. KING, H. A. MATTILL.

PAST OFFICERS

1933 L. B. MENDEL, President; H. C. SHERMAN, Vice-President; J. R. MURLIN, Secretary-Treasurer; E. F. DUBOIS, M. S. ROSE, Councilors. **1934** J. R. MURLIN, President; E. F. DUBOIS, Vice-President; ICIE G. MACY, Secretary; W. M. BOOTHBY, Treasurer; A. H. SMITH, AGNES FAY MORGAN, R. M. BETHKE, Councilors. **1935** J. R. MURLIN, President; E. F. DUBOIS, Vice-President; ICIE G. MACY, Secretary; G. R. COWGILL, Treasurer; A. H. SMITH, R. M. BETHKE, L. A. MAYNARD, Councilors. **1936** E. F. DUBOIS, President; MARY SWARTZ ROSE, Vice-President; G. R. COWGILL, Treasurer; ICIE G. MACY, Secretary; R. M. BETHKE, L. A. MAYNARD, C. A. ELVEHJEM, Councilors. **1937** MARY S. ROSE, President; E. V. MCCOLLUM, Vice-President; G. R. COWGILL, Treasurer; ICIE G. MACY, Secretary; L. A. MAYNARD, C. A. ELVEHJEM, P. E. HOWE, Councilors. **1938** E. V. MCCOLLUM, President; T. M. CARPENTER, Vice-President; G. R. COWGILL, Treasurer; L. A. MAYNARD, Secretary; C. A. ELVEHJEM, P. E. HOWE, HELEN S. MITCHELL, Councilors. **1939** H. C. SHERMAN, President; T. M. CARPENTER, Vice-President; G. R. COWGILL, Treasurer; L. A. MAYNARD, Secretary; P. E. HOWE, HELEN S. MITCHELL, A. H. SMITH, Councilors. **1940** THORNE M. CARPENTER, President; A. G. HOGAN, Vice-President; L. A. MAYNARD, Secretary; W. H. SEBRELL, JR., Treasurer; HELEN S. MITCHELL, ARTHUR H. SMITH, LYDIA J. ROBERTS, Councilors. **1941** A. G. HOGAN, President; L. A. MAYNARD, Vice-President; ARTHUR H. SMITH, Secretary; W. H. SEBRELL, JR., Treasurer; T. H. JUKES, LYDIA J. ROBERTS, H. B. LEWIS, Councilors.

CONSTITUTION

1. The name of the proposed society is the "AMERICAN INSTITUTE OF NUTRITION."

2. The purposes of the society are to further the extension of the knowledge of nutrition and to facilitate personal contact between investigators in nutrition and closely related fields of interest.

3. The management of the American Institute of Nutrition shall be vested in a council consisting of the President, Vice-President, Secretary, Treasurer and three additional members.

BY-LAWS

ARTICLE I—*Membership*

SECTION 1. There shall be two classes of members, members and emeritus members. The number of members shall be limited to 300 exclusive of emeritus members.

SEC. 2. *Eligibility for membership:* Members. Qualified investigators who have independently

conducted and published meritorious original investigations in some phase of the chemistry or physiology of nutrition and who have shown a professional interest in nutrition for at least 5 years shall be eligible for membership in the Society. Emeritus Members. Members in good standing who have reached the age of 65 years shall become emeritus members. A member in good standing and for sufficient reason may by vote of the Council be made an emeritus member. Emeritus members shall be entitled to vote but not hold office.

SEC. 3. *Nomination:* Nominations for membership shall be made and seconded by members of the Society on blanks furnished by the Secretary. Nominations shall be submitted to the Council who shall determine eligibility and make recommendation to the Society at a regular meeting.

SEC. 4. *Election to membership:* A. A nominee for membership may be voted for by ballot at any meeting of the Society after the Council has reported its findings on his eligibility. B. A majority of the ballots cast shall elect.

SEC. 5. *Forfeiture:* If a majority of the Council after due notice to the member in question and opportunity for a hearing, shall decide that the interests of the Society require the expulsion of a member, the Secretary shall send a notice of this decision to each member at least two weeks before the next annual meeting. At this meeting the Secretary shall, on behalf of the Council, propose the expulsion; and if two-thirds of the members present vote for it, the member shall be expelled, his assessment for the current year shall be returned to him, and he shall cease to be a member of the Society.

ARTICLE II—*Meetings and Quorum*

SECTION 1. *Annual:* The annual meeting of the Society shall be held on the date fixed by the Certificate of Incorporation.

SEC. 2. *Special:* A special meeting may be called at any time by the President, or in case of his absence or disability, by the Vice-President, and must be called at the request in writing of a majority of the Council or fifteen members of the Society. Notice specifying the purpose of such meeting shall be mailed to each member at least ten days previous thereto. The Council shall select the places at which meetings shall be held.

SEC. 3. *Quorum:* Fifteen members shall constitute a quorum at all meetings of the Society, but in absence of a quorum any number shall be sufficient to adjourn to a fixed date.

ARTICLE III—*Officials*

SECTION 1. *Officers:* The officers shall be a President, a Vice-President, a Secretary, and a Treasurer, who shall be elected annually by the members of the Society. Their terms of office shall commence on May 1 of the year in which they are elected.

SEC. 2. *Council:* The officers so selected and three additional members, one of whom shall be elected at each annual meeting to serve a term of three years, shall constitute a Board of Trustees and shall be known as 'The Council.' (When this provision is first put into effect one member shall be elected for 1 year, one for 2 years and the third for 3 years.)

SEC. 3. *Duties of Officers:* The powers and duties of the officers elected by the Society shall be such as usually devolve upon their respective positions.

ARTICLE IV—*The Council*

SECTION 1. *Powers:* The general management of the Society during the intervals between meetings shall be vested in the Council, which shall regularly perform the ordinary duties of an executive committee and possess all the powers conferred upon the Board of Trustees of an educational institution chartered by the Education Department of the University of the State of New York. A permanent charter was issued to the American Institute of Nutrition under date of November 16, 1934.

SEC. 2. *Reports:* The Council shall report to the Society its findings on the eligibility of candidates for membership, and on all charges of a violation of these By-Laws.

ARTICLE V—*Nominating Committee*

SECTION 1. *Membership:* A. The Nominating Committee shall consist of five members appointed for the coming year by the retiring President. Members who have served on the Nominating Committee for two consecutive years shall be ineligible for reappointment until after a lapse of one year. B. The President shall designate one member to be Chairman of the Nominating Committee.

SEC. 2. *Nomination of Officials:* A. The Nominating Committee shall make at least one nomination for each of the four offices, for each of the additional positions on the Council to be filled by vote of the members and for each of the positions on the Editorial Board to be vacated at the time of the annual meeting. Any member of the Institute may submit nominations to the Nominating Committee for its consideration along with those nominations made by the members of the Nominating Committee. B. The nominations by the Nominating Committee shall be transmitted to the Secretary at least six weeks before the annual meeting at which they are to be considered. C. The Secretary shall send to every member, at least two weeks before

the annual meeting, a printed ballot containing the list of nominees and space for such additional names as the member wishes to propose, and at the same time shall notify the members that they may vote by mail, returning to the Secretary the marked ballot in the envelope provided, at such a time and place as the Secretary may designate, or the ballot may be delivered to the Secretary at the beginning of the business session at which the elections are to take place.

SEC. 3. *Election of Officials:* A. At the beginning of the business session the Secretary shall present to the tellers, appointed by the President, the ballots submitted by the members and the ballots shall be counted forthwith. B. A majority of votes cast shall be necessary to elect an official.

SEC. 4. *Filling of Vacancies:* A. The Nominating Committee shall fill all vacancies in elective positions except such as may occur at a meeting of the Society. B. The President of the Society shall fill all vacancies in appointive positions.

ARTICLE VI—*Financial*

SECTION 1. *Dues:* The dues shall be the annual cost of subscription to The Journal of Nutrition for members plus an annual assessment which shall be determined by majority vote at the annual meetings, upon recommendation of the Council, and shall be due within a month after the annual meeting. Emeritus members are not required to pay assessments or to subscribe to The Journal of Nutrition.

SEC. 2. *Expenditures:* No expenditures from the general funds of the Society except those required in the performance of the ordinary official duties shall be made except by vote of the Society or the Council.

SEC. 3. *Penalty for non-payment of dues:* A. Members in arrears for dues for two consecutive years shall forfeit their membersip. B. Delinquent members may be reinstated by the Council provided all indebtedness to the Society is liquidated.

ARTICLE VII—*The Journal of Nutrition*

SECTION 1. The American Institute of Nutrition designates The Journal of Nutrition as its official organ of publication.

SEC. 2. In accordance with the expressed wish of the Wistar Institute of Anatomy and Biology, owner and publisher of The Journal of Nutrition, the American Institute of Nutrition shall nominate members of the Editorial Board for its official organ. A. The editorial management of The Journal of Nutrition shall be vested in an Editorial Board consisting of an Editor and twelve Board Members. B. The Editor shall be chosen by the Editorial Board to serve a term of five years beginning May 1 of the year in which he is chosen, and shall be eligible for reëlection. The Editor shall have the power to designate one of the Board Members to serve as his assistant, and such an appointee shall be called Associate Editor. C. Three members of the Institute shall be nominated by the Nominating Committee for membership on the Editorial Board each year to serve a term of four years, replacing three retiring members and taking office May 1 of the year in which they are elected. In the event of a vacancy in the membership of the Editorial Board occurring through death or other reason, the Nominating Committee, for each such vacancy to be filled shall make an additional nomination. In this event the nominees elected who receive the greatest number of votes shall serve the longest term of vacancies to be filled. D. Retiring members of the Editorial Board shall not be eligible for renomination until one year after their retirement.

ARTICLE VIII—*Papers on Scientific Subjects*

SECTION 1. The Secretary shall be authorized to arrange programs for the scientific sessions at the annual meetings.

ARTICLE IX—*Changes in Constitution and By-Laws*

SECTION 1. Proposed changes in the Constitution and By-Laws must be sent in writing to the Secretary at least one month before the date of the meeting at which they are to be considered, and must be signed by at least three members. The Secretary shall send a printed copy of any proposed change to each member at least two weeks before the next meeting and shall notify all members that they may vote by proxy.

SEC. 2. If at this meeting two-thirds of the votes cast shall favor the proposed change, it shall be made.

THE AMERICAN ASSOCIATION OF IMMUNOLOGISTS

Founded June 19, 1913; Member of Federation 1941

OFFICERS ELECTED 1942

President—JACQUES J. BRONFENBRENNER, Washington University School of Medicine, St. Louis, Mo.

Secretary-Treasurer—ARTHUR F. COCA, Pearl River, N. Y.

Council—JACQUES J. BRONFENBRENNER, ARTHUR F. COCA, MICHAEL HEIDELBERGER, 620 W. 168 St., New York City, PAUL R. CANNON, University of Chicago, Chicago, Ill., KARL F. MEYER, Medical Center, San Francisco, Cal., GEORGE P. BERRY, University of Rochester, Rochester, N. Y., DONALD T. FRASER, Connaught Laboratories, University of Toronto, Toronto, Canada, SANFORD B. HOOKER, (Ex officio), 80 East Concord St., Boston, Mass., JOHN F. ENDERS, (Ex officio), Harvard University School of Medicine, Boston, Mass.

PAST OFFICERS

Presidents—**1913** GERALD B. WEBB. **1915** JAMES W. JOBLING. **1916** RICHARD WEIL. **1917** JOHN A. KOLMER. **1918** WILLIAM H. PARK. **1919** HANS ZINSSER. **1920** RUFUS I. COLE. **1921** FREDERICK P. GAY. **1922** GEORGE W. MCCOY. **1923** H. GIDEON WELLS. **1924** FREDERICK G. NOVY. **1925** WILFRED H. MANWARING. **1926** LUDVIG HEKTOEN. **1927** KARL LANDSTEINER. **1928** EUGENE L. OPIE. **1929** OSWALD T. AVERY. **1930** STANHOPE BAYNE-JONES. **1931** ALPHONSE R. DOCHEZ. **1932** AUGUSTUS B. WADSWORTH. **1933** THOMAS M. RIVERS. **1934** FRANCIS G. BLAKE. **1935** WARFIELD T. LONGCOPE. **1936** SANFORD B. HOOKER. **1937** CARL TENBROECK. **1938** DONALD T. FRASER. **1939** GEORGE P. BERRY. **1940** PAUL R. CANNON. **1941** KARL F. MEYER.

Vice-Presidents—**1913–1915** GEORGE W. ROSS. **1915** GEORGE P. SANBORN. **1916** JOHN A. KOLMER.

Secretary—**1913–1918** MARTIN J. SYNNOTT.

Treasurer—**1913–1918** WILLARD J. STONE.

Secretary-Treasurer—**1918–*date*.** ARTHUR F. COCA.

CONSTITUTION AND BY-LAWS

Adopted April 6, 1917

ARTICLE I

SECTION 1. This Association shall be called "The American Association of Immunologists."

SEC. 2. The purpose of the Association shall be to study the problems of immunology and its application to clinical medicine.

ARTICLE II

SECTION 1. The Association shall be governed by a Council of seven, which shall consist of the officers of the association and enough active members to make a total of seven members.

SEC. 2. The officers of the Association shall be a President, a Secretary, and a Treasurer, who shall be nominated annually by the Council, and elected by the Society to serve for one year. Nominations of officers may be made also by members of the Society.

SEC. 3. No councilor is eligible for re-election until after one year, except the Secretary and the Treasurer, who are eligible for re-election.

SEC. 4. If any councilor without good and sufficient reason fails to attend two consecutive meetings of the Council he shall be considered to have resigned.

SEC. 5. The same person shall not serve as President more than one year consecutively.

SEC. 6. It is the duty of the Council to conduct the business of the Association and to elect the new members. Should a vacancy occur in the Council otherwise than by the expiration of the term of service, the Council may elect a member to serve for the unexpired portion of the term.

ARTICLE III

SECTION 1. Active Members. Any one actively engaged in the systematic study of problems relating to immunology shall be eligible to active membership.

ARTICLE IV

Candidates for membership shall be nominated by two active members of the Association who shall present in writing to the Council evidence of the fitness of the candidates to become members of the Association.

ARTICLE V

If a majority of the Council shall decide that the interests of the Society require the expulsion of a member, the Secretary shall send a notice of this decision to each active member at least two weeks before the next annual meeting. At this meeting the Secretary shall, on behalf of the Council, propose the expulsion; and if two-thirds of the members present vote for it, the member shall be expelled, his assessment for the current year shall be returned to him, and he shall cease to be a member of the Society.

Article VI

Section 1. A quorum of the Council for the transaction of all business shall be three.

Sec. 2. Any number of members present at the time appointed for the annual meeting of the Association, shall constitute a quorum.

BY-LAWS

1. A regular meeting of the Association shall be held annually at such time and place as the Council shall determine.

2. Special meetings of the Association may be held at the discretion of the Council.

3. These regular and special meetings shall be open to all members of the Association.

4. A meeting of the Council shall be held shortly before each annual session of the Association.

5. Hereafter each Councilor shall serve for a period of six years. Under this rule the service of one member and also that of the Secretary-Treasurer terminates at the meeting of 1936. At that meeting two members shall be elected to the Council, one of whom may serve as Secretary-Treasurer. Thereafter the period of service of these two members shall run concurrently; hence, two members must be elected to the Council every six years in order to maintain a membership of seven.

6. Past Presidents are honorary members of the Council.

7. The titles of all communications to be presented before the Association shall be approved by the Council.

8. Failure of an active member to offer a paper at least once in three years shall be equivalent to resignation. If in its judgment there is sufficient reason the Council may, in individual cases, suspend this rule.

9. The dues of the Association shall be fixed annually by the Council.

10. Failure to pay dues for three successive years shall constitute annulment of membership.

11. The constitution and by-laws may be amended by a two-thirds vote of the active members present at any regular meeting.

12. No amendment shall be adopted at the meeting at which it is proposed.

13. The Journal of Immunology, which is the property and official organ of this Association, shall be administered for the Association by an editorial staff to consist of an Editor-in-Chief and at least three Associate Editors, with the advice of a Board of Editors.

14. The members of the editorial staff shall be elected or may be removed by a majority vote of the Council of the Association.

ALPHABETICAL LIST OF ALL MEMBERS OF THE SIX SOCIETIES

The parenthesis following each listed name gives the Society affiliation and year of election:

(1) The American Physiological Society
(2) The American Society of Biological Chemists
(3) The American Society for Pharmacology and Experimental Therapeutics
(4) The American Society for Experimental Pathology
(5) The American Institute of Nutrition
(6) The American Association of Immunologists

HONORARY MEMBERS

Castaneda, M. Ruiz, M.D. Investigaciones Medicas, Hospital General, Mexico, D. F. *Director, Department of Medical Research.* (6, 1942)

Chopra, R. N., M.A., M.D., Sc.D.(Cantab), F.R.C.P. (London) P.I.E. School of Tropical Medicine, Calcutta, India. *Director; Professor of Pharmacology.* (3, 1938)

Dale, H. H. Medical Research Council, National Institute for Medical Research, Hampstead, London, N.W. 3, England. *Director, National Institute for Medical Research.* (3, 1926)

Flexner, Simon, M.D., Sc.D.(hon.), LL.D. 520 E. 86th St., New York City. *Emeritus Director, Rockefeller Institute for Medical Research.* (6, 1920)

Hektoen, Ludvig, M.D. 629 S. Wood St., Chicago, Ill. *President, Chicago Tumor Institute.* (6, 1919)

Hitchens, Arthur P., M.D. Medical School, University of Pennsylvania, Philadelphia. *Professor of Public Health and Preventive Medicine; Lt. Col., M.C., U.S.A.* (6, 1913)

Houssay, Bernardo A., M.D. Facultad de Medicina, Universidad de Buenos Aires, Instituto de Fisiologia, Buenos Aires, Argentina. *Professor of Physiology and Director.* (1, 1942)

Huntoon, F. M., M.D. Woodbridge, Conn. **(6,** 1918)

Koller, Carl, M.D. 30 W. 58th St., New York City. **(3,** 1930)

Lowei, Otto, M.D. New York University College of Medicine, 477 First Ave., New York City. *Research Professor in Pharmacology.* **(3,** 1941)

McCoy, George Walter, M.D. Louisiana State University Medical School, New Orleans. *Director, Department of Public Health.* **(6,** 1916)

Novy, Frederick G., M.D., Sc.D., LL.D. 721 Forest Ave., Ann Arbor, Mich. *Dean Emeritus and Professor Emeritus of Bacteriology, Medical School, University of Michigan.* **(6,** 1920)

Rosenau, Milton J., M.D., A.M. Medical School, University of North Carolina, Chapel Hill. *Director, School of Public Health; Professor of Epidemiology, School of Public Health.* **(6,** 1918)

Sherrington, Sir Charles S., O.M., Sc.D., M.D., F.R.S. "Broomside," Valley Road, Ipswich, England. *Former Wayneflete Professor of Physiology, Oxford University; Former President of the Royal Society.* **(1,** 1904)

Sordelli, A. Institute of Bacteriology, Department of Public Health, Buenos Aires, Argentina. *Director.* **(6,** 1942)

Straub, Walther, M.D. University of Munich, Germany. **(3,** 1927)

Wells, H. Gideon, A.M., M.D., Ph.D. University of Chicago, Chicago, Ill. *Emeritus Professor of Pathology, University of Chicago; Emeritus Director of the Otho S. A. Sprague Memorial Institute.* **(6,** 1918)

MEMBERS

Abramson, David Irwin, M.D. The May Institute for Medical Research, The Jewish Hospital, Cincinnati, O. *Director of the Cardiovascular Laboratory.* **(1,** 1937)

Abramson, Harold A., M.D. 133 E. 58th St., New York City. *Assistant Professor of Physiology, College of Physicians and Surgeons, Columbia University.* **(1,** 1930; **2,** 1934)

Abreu, Benedict E., M.S., Ph.D. University of Georgia School of Medicine, Augusta. *Assistant Professor of Pharmacology.* **(3,** 1941)

Acheson, George H., M.D. Harvard Medical School, 25 Shattuck St., Boston, Mass. *Instructor in Physiology.* **(1,** 1942)

Adams, Mildred, M.A., Ph.D. Takamine Laboratory, Clifton, N. J. *Research Chemist.* **(2,** 1934)

Adams, R. Charles, M.D., C.M., M.S. (Anesthesiology), Mayo Clinic, Rochester, Minn. *Instructor in Anesthesia, Mayo Foundation, University of Minnesota. Member of Mayo Clinic Staff, Section on Anesthesia.* **(3,** 1942)

Adams, W. Lloyd, M.A., Ph.D. Albany Medical College, Albany, N. Y. *Assistant Professor of Physiology and Pharmacology.* **(3,** 1942)

Addis, Thomas, M.D., M.R.C.P. Lane Hospital, San Francisco, Calif. *Professor of Medicine, Stanford University.* **(1,** 1922)

Addison, William H. F., M.D. School of Medicine, University of Pennsylvania, Philadelphia. *Professor of Histology and Embryology.* **(1,** 1928)

Adolph, Edward Frederick, Ph.D. School of Medicine and Dentistry, University of Rochester, Rochester, N. Y. *Associate Professor of Physiology.* **(1,** 1921)

Adolph, William Henry, Ph.D. Yenching University, Peiping, China. *Professor of Biochemistry.* **(5,** 1934)

Albritton, Errett C., M.D. George Washington University Medical School, 1339 H St., N.W., Washington, D. C. *Professor of Physiology and Head of the Department of Physiology.* **(1,** 1933)

Allen, Frank N., M.D. Lahey Clinic, 605 Commonwealth Ave., Boston, Mass. *Co-director of the Medical Department.* **(4,** 1930)

Allen, Frederick M., M.D. 1031 Fifth Ave., New York City. *Professor of Medicine, Polyclinic Medical School and Hospital.* **(1,** 1924; **4,** prior to 1920)

Allen, Lane, M.S., Ph.D., M.D. University of Georgia School of Medicine, University Place, Augusta. *Associate Professor of Anatomy.* **(1,** 1939)

Allen, Willard M., M.D. Washington University School of Medicine, 630 S. Kingshighway Blvd., St. Louis, Mo. *Professor of Obstetrics and Gynecology.* **(1,** 1934)

Allen, William F., Ph.D. University of Oregon Medical School, Portland. *Professor of Anatomy.* **(1,** 1929)

Alles, Gordon A., M.S., Ph.D. 770 S. Arroyo Parkway, Pasadena, Calif. *Lecturer in Pharmacology, University of California Medical School, San Francisco, and Research Associate in Biology, California Institute of Technology, Pasadena.* **(1,** 1932; **3,** 1941)

Almquist, Herman J., Ph.D. University of California, Division of Poultry Husbandry, Berkeley. *Associate Professor of Poultry Husbandry.* **(2,** 1937; **5,** 1937)

Alvarez, Walter C., M.D. Mayo Clinic, Rochester, Minn. *Professor of Medicine, Mayo Foundation.* **(1,** 1917; **3,** 1921)

Alving, Alf Sven, M.D. Billings Hospital, University of Chicago, 950 E. 59th St., Chicago, Ill. *Associate Professor of Medicine.* **(1,** 1939)

Amberg, Samuel, M.D., F.A.A.P. Mayo Clinic, Rochester, Minn. *Associate Professor of Pediatrics, Mayo Foundation, University of Minnesota.* **(1,** 1903; **2,** 1906; **3,** 1909)

Amberson, William R., Ph.D. University of Maryland School of Medicine, Baltimore. *Professor of Physiology.* (1, 1924)

Ambrose, Anthony M., M.S., Ph.D. University of Louisville School of Medicine, Louisville, Ky. *Associate Professor of Pharmacology.* (3, 1937)

Amoss, Harold L., M.D., M.S., D.P.H., Sc.D. 21 Field Point Road, Greenwich, Conn. (4, 1922; 6, 1917)

Andersch, Marie A., M.S., Ph.D. Children's Hospital, DeSoto St., Pittsburgh, Pa. *Research Fellow, University of Pittsburgh.* (2, 1940)

Andersen, Dorothy H., M.D. Babies Hospital, Broadway and 167th St., New York City. *Associate in Pathology, Columbia University.* (4, 1935)

Anderson, Evelyn M., M.A., M.D. University of California Hospital, San Francisco. *Assistant Professor of Medicine.* (1, 1934)

Anderson, Hamilton H., M.S., M.D. 1405 Drake Ave., Burlingame, Calif. *Professor of Pharmacology, Peiping Union Medical College.* (3, 1931)

Anderson, Oscar Daniel, Ph.D. Stimson Hall, Cornell University, Ithaca, N. Y. *Assistant Professor of Physiology.* (1, 1939)

Anderson, Rudolph J., Ph.D. Sterling Laboratory, Yale University, New Haven, Conn. *Professor of Chemistry.* (2, 1915)

Anderson, W. A. D., M.A., M.D. St. Louis University School of Medicine, St. Louis, Mo. *Assistant Professor of Pathology.* (4, 1941)

Anderson, William E., M.A. Eastern State Farmers' Exchange, Westbrook Farm, Rockville, Conn. *Biochemist.* (2, 1931; 5, 1933)

Andervont, H. B., Sc.D. National Cancer Institute, Bethesda, Md. *Principal Biologist, U. S. Public Health Service.* (4, 1939)

Andrews, James C., Ph.D. University of North Carolina, Chapel Hill. *Professor of Biological Chemistry.* (2, 1925)

Andrus, E. Cowles, M.D. Johns Hopkins Hospital, Baltimore, Md. *Assistant Visiting Physician; Associate Professor of Medicine, Johns Hopkins University.* (1, 1925)

Angevine, D. Murray, M.D. Alfred I. du Pont Institute, Wilmington, Del. *Pathologist; Visiting Assistant Professor of Pathology, University of Pennsylvania.* (4, 1940)

Angier, Roswell Parker, Ph.D. % Los Rauchos Perkins, Tucson, Ariz. *Professor of Psychology, Yale University.* (1, 1906)

Ansbacher, Stefan, M.S., D.Sc. Research Laboratory, American Home Products Corp., 84–48 129th St., Richmond Hill, L. I., N. Y. *Director of Research.* (2, 1939)

Anson, Mortimer L., Ph.D. The Rockefeller Institute for Medical Research, Princeton, N. J. *Associate.* (2, 1937)

Apfelbach, Carl Wesley, M.D. Presbyterian Hospital, 1753 W. Congress St., Chicago, Ill. *Rush Professor of Pathology, University of Illinois.* (4, 1937)

Apperly, Frank L., M.D. Medical College of Virginia, Richmond. *Professor of Pathology.* (4, 1936)

Arkin, Aaron, M.A., M.D., Ph.D. Suite 2006, 25 E. Washington St., Chicago, Ill. *Associate Professor of Medicine, Rush Medical College, Univ. of Chicago; Professor and Chairman, Dept. of Medicine, Cook County Graduate School.* (1, 1914; 3, 1919)

Armstrong, W. D., M.S., M.D., Ph.D. Medical Sciences Bldg., University of Minnesota, Minneapolis. *Associate Professor of Physiological Chemistry.* (2, 1938)

Arnold, Lloyd, A.M., M.D. 1538 E. 57th St., Chicago, Ill. *Professor of Bacteriology and Preventive Medicine, Illinois College of Medicine; Bacteriologist, State Department of Public Health.* (4, 1930; 6, 1925)

Arnow, L. Earle, Ph.D., M.D. Medical Research Division, Sharp and Dohme, Glenolden, Pa. *Director of Biochemical Research.* (2, 1940)

Aronson, Joseph D., M.D. Hospital, Fort Belvoir, Va. *Associate Professor of Bacteriology, Phipps Institute, University of Pennsylvania; Major, M. C., U. S. A.* (4, 1927; 6, 1925)

Ascham, Leah, Ph.D. Kansas State College, Manhattan. *Associate Professor, School of Home Economics.* (5, 1935)

Ashby, Winifred M., Ph.D. 305 10th St., N.E., Washington, D. C. *Senior Scientist, Federal Security Agency (St. Elizabeth's Hospital).* (6, 1923)

Ashman, Richard, M.S., Ph.D. School of Medicine, Louisiana State University, New Orleans. *Professor of Physiology.* (1, 1925)

Astwood, Edwin Bennet, M.D., C.M., Ph.D. Harvard Medical School, 25 Shattuck St., Boston, Mass. *Assistant Professor of Pharmacotherapy.* (1, 1939)

Aub, Joseph C., M.D. Collis P. Huntington Memorial Hospital, 695 Huntington Ave., Boston, Mass. *Associate Professor of Medicine, Harvard Medical School.* (1, 1919; 5, 1933)

Auer, John, M.D. 1402 S. Grand Blvd., St. Louis, Mo. *Professor of Pharmacology and Director of the Department, St. Louis University School of Medicine.* (1, 1905; 3, 1908)

Austin, J. Harold, M.D. 711 Maloney Clinic, 36th and Spruce Sts., Philadelphia, Pa. *Director, William Pepper Laboratory of Clinical Medicine.* (2, 1922)

Austin, Richard Sisson, M.D. Cincinnati General Hospital, University of Cincinnati, Cincinnati, O. *Professor of Pathology.* (4, 1927)

Avery, O. T., M.D., Sc.D., LL.D. Hospital of the Rockefeller Institute, 66th St. and York Ave., New York City. *Member, Rockefeller Institute for Medical Research.* (**4,** 1921; **6,** 1920)

Axtmayer, Joseph H., A.M., Ph.D. School of Tropical Medicine, San Juan, Porto Rico. *Associate Professor of Chemistry.* (**5,** 1935)

Babkin, B. P., M.D., D.Sc., F.R.S.C. McGill University, Montreal, Canada. *Professor of Physiology.* (**1,** 1924)

Bachem, Albert, Ph.D. College of Medicine, University of Illinois, 1853 W. Polk St., Chicago. *Professor of Biophysics.* (**1,** 1933)

Bachman, Carl, M.D. University Hospital, 3400 Spruce St., Philadelphia, Pa. *Professor of Obstetrics and Gynecology.* (**2,** 1941)

Bachmann, George, M.S., M.D., F.A.C.P. 1088 Lullwater Road, Atlanta, Ga. *Professor of Physiology, Emory University School of Medicine.* (**1,** 1912)

Baer, Erich, Ph.D. Banting Institute, 100 College St., Toronto, Canada. *Assistant Research Professor of Organic Chemistry, University of Toronto.* (**2,** 1942)

Baernstein, Harry D., M.S., Ph.D. National Institute of Health, Bethesda, Md. *Associate Biochemist.* (**2,** 1934)

Baetjer, Anna M., D.Sc. Johns Hopkins School of Hygiene and Public Health, 615 N. Wolfe St., Baltimore, Md. *Associate in Physiology.* (**1,** 1929)

Bahrs, Alice M., M.A., Ph.D. The Martha Washington Hotel, 10th and Montgomery Sts., Portland, Ore. *Research Associate in Nutrition, University of Oregon.* (**1,** 1933)

Bailey, Cameron Vernon, M.D., C.M. 303 E. 20th St., New York City. *Professor of Clinical Medicine, New York Post-Graduate Medical School, Columbia University.* (**2,** 1920; **5,** 1933)

Bailey, Orville T., M.D. Harvard University Medical School, 25 Shattuck St., Boston, Mass. *Associate in Pathology; Associate Pathologist, Peter Bent Brigham Hospital.* (**4,** 1939)

Bailey, Percival, M.D., Ph.D. University of Illinois College of Medicine, 912 S. Wood St., Chicago. *Professor of Neurology and Neurosurgery.* (**1,** 1941)

Baitsell, George Alfred, A.M., Ph.D. Yale Station, New Haven, Conn. *Professor of Biology, Yale University.* (**1,** 1915)

Baker, A. B., M.D. University of Minnesota Medical School, 126 Millard Hall, Minneapolis. *Associate Professor of Neuropsychiatry and Neuropathology.* (**4,** 1940)

Baker, Roger D., M.D. Duke Hospital, Durham, N. C. *Assistant Professor of Pathology, Duke University Medical School; Assistant Pathologist, Duke Hospital.* (**4,** 1939)

Baldes, Edward J., A.M., Ph.D. Mayo Foundation, Rochester, Minn. *Assistant Professor of Physics, Mayo Foundation, Graduate School, University of Minnesota.* (**1,** 1930)

Baldwin, Francis Marsh, A.M., Ph.D. University of Southern California, Los Angeles. *Professor of Zoology and Director of Experimental Marine Biology.* (**1,** 1919)

Ball, Eric G., M.A., Ph.D. Harvard Medical School, Boston, Mass. *Associate Professor of Biochemistry.* (**2,** 1934)

Ball, Howard A., M.D. San Diego County General Hospital, N. Front St., San Diego, Calif. *Pathologist, San Diego County General and Paradise Valley Hospitals.* (**4,** 1937)

Balls, Arnold Kent, Ph.D. U. S. Bureau of Agricultural Chemistry and Engineering, Washington, D. C. *Head Chemist, Enzyme Research Laboratory; Adjunct Professor, The George Washington University.* (**2,** 1932)

Banus, Mario Garcia, M.Sc., D.Sc. Tufts College Medical School, Boston, Mass. *Associate Professor of Physiology.* (**1,** 1927)

Barbour, Henry Gray, M.D. Yale University School of Medicine, 333 Cedar St., New Haven, Conn. *Research Associate and Professor of Pharmacology.* (**1,** 1912; **2,** 1928; **3,** 1912)

Bard, Philip, A.M., Ph.D. Johns Hopkins University School of Medicine, 710 N. Washington St., Baltimore, Md. *Professor of Physiology.* (**1,** 1929)

Barkan, Georg, M.D., Dr. habil. 80 E. Concord St., Boston, Mass. *Former Professor of Pharmacology and Director of the Pharmacological Institute, Univ. of Dorpat (Estonia); Assistant Professor of Biochemistry, Boston University School of Medicine.* (**3,** 1939)

Barker, S. B., Ph.D. University of Tennessee, Memphis. *Instructor in Physiology.* (**1,** 1938)

Barlow, Orpheus W., M.D., Ph.D. Winthrop Chemical Co., 33 Riverside Ave., Rensselaer, N. Y. *Director of Biologic and Research Laboratories.* (**1,** 1936)

Barnes, B. O., A.M., Ph.D. 2220 S. St. Paul, Denver, Colo. *Professor of Health Education, University of Denver.* (**1,** 1932)

Barnes, LaVerne A., M.S., Ph.D. 7 Maple Ave., Bethesda, Md. *Lieutenant, H-V(S), U.S.N.R. (Epidemiology and Sanitation Unit, National Naval Medical School).* (**6,** 1931)

Barnes, Richard Henry, Ph.D. Department of Physiology, University of Minnesota, Minneapolis. *Assistant Professor of Physiological Chemistry.* (**2,** 1941)

Barnes, Thomas C., D.Sc. Oglethorpe University, Atlanta, Ga. *Professor of Pharmacology.* (**1,** 1942)

Barott, Herbert G., E.E. U. S. Department of Agriculture, National Agricultural Research

Center, Beltsville, Md. *Biophysicist, Animal Nutrition Division, Bureau of Animal Industry.* (5, 1938)

Barrera, S. Eugene, M.D. Psychiatric Institute and Hospital, Columbia University, 722 W. 168th St., New York City. *Principal Research Psychiatrist and Assistant Professor of Psychiatry.* (1, 1937)

Barron, E. S. Guzman, M.D. Department of Medicine, University of Chicago, Chicago, Ill. *Assistant Professor of Biochemistry.* (2, 1931)

Bartley, S. Howard, Ph.D. Dartmouth Eye Institute, Dartmouth College, Hanover, N. H. *Assistant Professor of Research in Physiological optics.* (1, 1935)

Batchelder, Esther L., A.M., Ph.D. Rhode Island State College, School of Agriculture and Home Economics, Kingston. *Head of Department of Home Economics.* (5, 1933)

Bates, Robert W., Ph.D. Difco Laboratories, Inc., 920 Henry St., Detroit, Mich. *Biochemist.* (2, 1936)

Batterman, Robert C., M.D. New York University College of Medicine, 477 First Ave., New York City. *Instructor in Therapeutics.* (3, 1941)

Baudisch, Oskar, Ph.D. Saratoga Springs, N. Y. *Director of Research, Saratoga Springs Authority, State of New York.* (2, 1931)

Bauer, Johannes H., M.D. Rockefeller Foundation, 49 W. 49th St., New York City. *Member of Staff, International Health Division of the Rockefeller Foundation.* (4, 1935)

Bauer, Walter, M.D. Massachusetts General Hospital, Boston. *Associate Professor and Tutor in Medicine, Harvard Medical School.* (1, 1929)

Bauman, Louis, M.D. Presbyterian Hospital, New York City. *Assistant Professor of Clinical Medicine, Columbia University.* (2, 1912)

Baumann, Carl A., M.S., Ph.D. Biochemistry Building, University of Wisconsin, Madison. *Associate Professor.* (2, 1938; 5, 1938)

Baumann, Emil J., Ph.D. 45 Lynwood Rd., Scarsdale, N. Y. *Chemist, Montefiore Hospital.* (2, 1922)

Baumberger, J. Percy, M.S., Sc.D. Stanford University, Calif. *Professor of Physiology.* (1, 1921)

Bayne-Jones, Stanhope, M.D. Yale University, School of Medicine, New Haven, Conn. *Professor of Bacteriology.* (4, 1927; 6, 1917)

Bazett, Henry C., M.A., M.D., F.R.C.S. Banting Institute, Toronto, Canada. *Professor of Physiology, University of Pennsylvania School of Medicine* (On leave). (1, 1921)

Beach, Eliot F., Ph.D. 660 Frederick St., Detroit, Mich. *Research Associate, Children's Fund of Michigan Research Laboratory.* (2, 1941; 5, 1942)

Bean, John W., M.S., Ph.D., M.D. University of Michigan, Ann Arbor. *Associate Professor of Physiology.* (1, 1932)

Beard, Howard H., M.A., Ph.D. 1542 Tulane Ave., New Orleans, La. *Professor of Biochemistry, Louisiana State University Medical Center.* (2, 1928; 5, 1933)

Beard, Joseph W., M.D. Duke University, Durham, N. C. *Assistant Professor of Surgery.* (4, 1938; 6, 1940)

Beazell, James Myler, Ph.D., M.D. Northwestern University Medical School, 303 E. Chicago Ave., Chicago, Ill. *Instructor in Physiology and Pharmacology.* (1, 1939)

Beck, Claude S., M.D. Lakeside Hospital, Cleveland, O. *Professor of Neurosurgery, Western Reserve University; Associate Surgeon, Lakeside Hospital.* (4, 1930)

Beck, Lyle V., M.S., Ph.D. Hahnemann Medical College, 235 N. 15th St., Philadelphia, Pa. *Instructor in Physiology.* (1, 1941)

Becker, Ernestine, M.A. Johns Hopkins University, Baltimore, Md. *Associate in Biochemistry.* (5, 1938)

Becker, R. Frederick, M.S., Ph.D. Northwestern University Medical School, 303 E. Chicago Ave., Chicago, Ill. *Instructor in Anatomy.* (1, 1941)

Beckman, Harry, M.D. Marquette University School of Medicine, Milwaukee, Wis. *Professor and Director of the Department of Pharmacology.* (3, 1937)

Beecher, Henry K., M.D. Massachusetts General Hospital, Boston. *Dorr Professor of Research in Anaesthesia, Harvard Medical School; Anesthetist-in-Chief, Massachusetts General Hospital.* (3, 1940)

Behre, Jeanette Allen, Ph.D. Department of Biochemistry, College of Physicians and Surgeons, 630 W. 168th St., New York City. *Visiting Scholar.* (2, 1925)

Belding, David L., M.D. Boston University School of Medicine, Boston, Mass. *Professor of Bacteriology and Experimental Pathology.* (4, 1927)

Bell, E. T., M.D. 110 Anatomy Bldg., University of Minnesota, Minneapolis. *Professor of Pathology.* (4, 1931)

Benedict, Francis Gano, Ph.D., Sc.D., M.D. Machiasport, Me. *Former Director of the Nutrition Laboratory of the Carnegie Institution of Washington; Member of the National Academy of Sciences.* (1, 1904; 2, 1906; 5, 1933)

Bennett, A. Lawrence, Ph.D., M.D. College of Medicine, University of Nebraska, Omaha. *Professor of Physiology and Pharmacology.* (1, 1941)

Bennett, Granville A., M.D. Harvard Medical School, 25 Shattuck St., Boston, Mass. *Assistant Professor of Pathology.* (4, 1931)

Bennett, Mary Adelia, M.A., Ph.D. Lankenau Hospital Research Institute, Philadelphia, Pa. *Research Biochemist.* (2, 1941)

Benson, Clara C., Ph.D. 157 Bloor St., W., Toronto, Canada. *Professor of Food Chemistry, University of Toronto.* (2, 1906)

Berg, Benjamin N., M.D. 630 W. 168th St., New York City. *Associate in Pathology, Columbia University, College of Physicians and Surgeons.* (4, 1928)

Berg, Clarence P., M.A., Ph.D. Chemistry Department, State University of Iowa, Iowa City. *Associate Professor of Biochemistry.* (2, 1933; 5, 1936)

Berg, William N., Ph.D. 789 West End Ave., New York City. *Biochemist.* (2, 1906)

Bergeim, Olaf, M.S., Ph.D. 1853 W. Polk St., Chicago, Ill. *Associate Professor of Physiological Chemistry, University of Illinois College of Medicine.* (1, 1916; 2, 1914)

Bergmann, Max, Ph.D. Rockefeller Institute for Medical Research, 66th St. and York Ave., New York City. *Member.* (2, 1934)

Bergmann, Werner, Ph.D. Sterling Chemistry Building, Yale University, New Haven, Conn. *Assistant Professor.* (2, 1934)

Berkson, Joseph, M.A., M.D., D.Sc. Mayo Clinic, Rochester, Minn. *Associate Professor, Biometry and Medical Statistics, Mayo Foundation, University of Minnesota.* (1, 1933)

Bernheim, Frederick, Ph.D. Box 3109, Duke Medical School, Durham, N. C. *Associate Professor of Physiology and Pharmacology.* (2, 1933; 3, 1935)

Bernthal, Theodore G., M.S., M.D. Vanderbilt University School of Medicine, Nashville, Tenn. *Assistant Professor of Physiology.* (1, 1932)

Berry, George Packer, M.D. University of Rochester, Rochester, N. Y. *Assistant Dean; Professor of Bacteriology; Associate Professor of Medicine.* (4, 1938; 6, 1934)

Bessey, Otto A., Ph.D. Public Health Research Institute of the City of New York, Park Laby., foot of E. 15th St. *Chief, Division of Nutrition and Physiology.* (2, 1938)

Best, Charles Herbert, M.A., M.D., D.Sc. (London), D.Sc. (Chicago), F.R.S. University of Toronto, Toronto, Ont., Canada. *Professor and Head of the Department of Physiology and the Banting-Best Department of Medical Research.* (1, 1923; 2, 1923)

Bethell, Frank H., M.D. 409 Lenawee Drive, Ann Arbor, Mich. *Assistant Professor of Internal Medicine and Assistant Director of the Thomas Henry Simpson Memorial Institute.* (4, 1936)

Bethke, Roland M., M.S., Ph.D. Ohio Agricultural Experiment Station, Wooster. *In Charge of Nutritional Investigations.* (2, 1928; 5, 1933)

Beutner, R., M.D., Ph.D. 235 N. 15th St., Philadelphia, Pa. *Professor and Head of Department of Pharmacology, Hahnemann Medical College.* (1, 1924; 3, 1924)

Beyer, Karl H., Jr., Ph.M., Ph.D. University of Wisconsin Medical School, Madison. *Instructor in Physiology.* (1, 1942)

Bieter, Raymond N., M.D., Ph.D. University of Minnesota, Minneapolis. *Professor of Pharmacology.* (3, 1930)

Bills, Charles E., M.A., Ph.D. Mead Johnson & Co., Evansville, Ind. *Director of Research.* (2, 1928; 5, 1935)

Bing, Franklin C., Ph.D. 535 N. Dearborn St., Chicago, Ill. *Secretary of Council on Foods, American Medical Assn.; Assistant Professor of Physiology, Northwestern University Medical School.* (2, 1931; 5, 1934)

Bing, Richard J., M.D. New York University College of Medicine, 477 First Ave., New York City. *Instructor in Physiology.* (1, 1942)

Binger, Carl A., M.D. 125 E. 73rd St., New York City. *Assistant Professor of Clinical Medicine (Psychiatry), Cornell University Medical College.* (1, 1927)

Binkley, Stephen Bennett, M.S., Ph.D. Research Department, Parke, Davis & Co., Detroit, Mich. (2, 1941)

Bisbey, Bertha, A.M., Ph.D. Gwynn Hall, University of Missouri, Columbia. *Professor of Home Economics.* (5, 1933)

Bischoff, Fritz E., M.S., Ph.D. Cottage Hospital, Santa Barbara, Calif. *Director of Research.* (2, 1928; 5, 1933)

Bishop, George H., Ph.D. Washington University Medical School, Euclid and Kingshighway, St. Louis, Mo. *Professor of Bio-Physics.* (1, 1923)

Blair, Edgar A., M.S., Ph.D. Washington University School of Medicine, 4580 Scott Ave., St. Louis, Mo. *Assistant Professor in Physiology.* (1, 1936)

Blair, Henry A., M.Sc., Ph.D. University of Rochester School of Medicine and Dentistry, Rochester, N. Y. *Associate Professor of Physiology.* (1, 1934)

Blake, Francis G., M.D., M.A. (hon.), Sc.D. Yale University School of Medicine, New Haven, Conn. *Dean and Sterling Professor of Medicine.* (4, prior to 1920; 6, 1921)

Blankenhorn, M. A., M.D. University of Cincinnati, Cincinnati, O. *Professor of Medicine.* (4, 1932)

Blatherwick, Norman R., M.S., Ph.D., Sc.D. Metropolitan Life Ins. Co., 1 Madison Ave.,

New York City. *Director of Biochemical Laboratory.* (1, 1915; 2, 1915; 5, 1934)

Blau, Nathan F., Ph.D. 1300 York Ave., New York City. (2, 1928)

Bliss, Eleanor A., Sc.D. Department of Preventive Medicine, Johns Hopkins Hospital, 615 N. Wolfe St., Baltimore, Md. *Associate in Preventive Medicine, Johns Hopkins University, School of Medicine.* (6, 1931)

Bliss, Sidney, Ph.D. Tulane University, New Orleans, La. *Professor of Biochemistry, School of Medicine.* (2, 1928)

Block, Richard Joseph, Ph.D. New York State Psychiatric Institute, 722 W. 168th St., New York City. *Associate Research Chemist.* (2, 1934; 5, 1933)

Block, Walter D., M.S., Ph.D. University Hospital, Ann Arbor, Mich. *Instructor in Biological Chemistry, Rackham Arthritis Research Unit.* (2, 1942)

Bloom, William, M.D. 1419 E. 56th St., Chicago, Ill. *Professor of Anatomy, University of Chicago.* (4, 1930)

Bloomfield, A. L., M.D. Stanford University Hospital, San Francisco, Calif. *Professor of Medicine.* (3, 1927; 4, 1927)

Bloor, W. R., A.M., Ph.D. School of Medicine and Dentistry, University of Rochester, Rochester, N. Y. *Professor of Biochemistry.* (1, 1915; 2, 1910)

Blum, Harold F., Ph.D. National Cancer Institute, National Institute of Health, U. S. Public Health Service, Bethesda, Md. *Senior Pharmacologist.* (1, 1928)

Blumberg, Harold, D.Sc. The Johns Hopkins University, Baltimore, Md. *Research Biochemist.* (5, 1942)

Blumenstock, Julius, M.D. Station Hospital, Fort Baker, Calif. *Captain, Medical Corps.* (1, 1925)

Blumgart, Herrmann L., M.D. Beth Israel Hospital, 330 Brookline Ave., Boston, Mass. *Associate Professor of Medicine, Harvard Medical School.* (1, 1927)

Blunt, Katharine, Ph.D., LL.D. Connecticut College, New London. *President, Connecticut College for Women.* (2, 1921)

Bock, Joseph C., Ch.E., Ph.D. 561 N. 15th St., Milwaukee, Wis. *Professor of Biochemistry, Marquette University Medical School.* (2, 1916)

Bodansky, Aaron, Ph.D. Hospital for Joint Diseases, 1919 Madison Ave., New York City. *Biological Chemist.* (2, 1926)

Bodansky, Oscar, Ph.D., M.D. Department of Pharmacology, Cornell University Medical College, New York City. *Lecturer in Biochemistry, Graduate Division, Brooklyn College; Assistant Adjunct Pediatrician, Beth Israel Hospital, New York.* (2, 1937; 3, 1942)

Bodine, Joseph Hall, Ph.D. State University of Iowa, Iowa City. *Professor and Head of Department of Zoology.* (1, 1925)

De Bodo, Richard C., M.D. 477 First Ave., New York City. *Associate Professor of Pharmacology, New York University College of Medicine.* (1, 1932; 3, 1931)

Boell, Edgar J., Ph.D. Osborn Zoological Laboratory, Yale University, New Haven, Conn. *Associate Professor of Biology.* (1, 1942)

Bogert, Marston T., Ph.D., LL.D., Sc.D., R.N.D. Chandler Laboratories, Columbia University, New York City. *Professor Emeritus of Organic Chemistry, in Residence; Member of the National Academy of Sciences.* (2, 1925)

Bolliger, Adolph, Ph.D. Gordon Craig Research Laboratories, University of Sydney, Sydney, Australia. *Director of Research.* (2, 1928)

Bollman, J. L., M.D. Mayo Clinic, Rochester, Minn. *Associate in Division of Experimental Surgery and Pathology, Mayo Clinic; Associate Professor of Experimental Pathology, Mayo Foundation, University of Minnesota.* (4, 1927)

Bond, Glenn C., Ph.D., M.D. The Upjohn Co., Research Laboratories, Kalamazoo, Mich. (6, 1939)

Booher, Lela E., M.S., Ph.D. Milwaukee Children's Hospital, Milwaukee, Wis. *Director, Institute of Nutrition.* (2, 1933; 5, 1933)

Bookman, Samuel, M.A., Ph.D. 624 Madison Ave., New York City. *Consulting Chemist, Mt. Sinai Hospital.* (2, 1912)

Boor, Alden K., Ph.D. Department of Medicine, University of Chicago, Chicago, Ill. *Assistant Professor of Biochemistry.* (2, 1931)

Boothby, W. M., M.D., M.A., F.A.C.S. Metabolism Laboratory, The Mayo Clinic, Rochester, Minn. *Chief of Section of Clinical Metabolism in Division of Medicine, Mayo Clinic; Professor of Experimental Metabolism, Mayo Foundation, University of Minnesota; Chairman, Mayo Aero-Medical Unit.* (1, 1915; 2, 1920; 3, 1923; 4, 1924; 5, 1933)

Bordley, James, III, M.D. Johns Hopkins Hospital, Baltimore, Md. *Associate Professor of Medicine, Johns Hopkins University.* (1, 1938)

Borsook, Henry, M.D., Ph.D. California Institute of Technology, Pasadena. *Professor of Biochemistry.* (2, 1931)

Bosworth, Alfred Willson, A.M., M.D. R. D. 4, Circleville, O. *Consulting Chemist.* (2, 1936; 5, 1935)

Bott, Phyllis A., M.S., Ph.D. Woman's Medical College of Pennsylvania, East Falls, Philadelphia. *Associate Professor of Physiological Chemistry.* (2, 1938)

Bourne, Wesley, M.D., C.M., M.Sc., F.R.C.P., D.A. (R.C.P. & S., Eng.). McGill University, Montreal, Canada. *Lecturer in Anesthetics,*

Dept. of Pharmacology and Therapeutics. (3, 1936)

Bourquin, Helen, M.S., Ph.D. 923 N. Nevada Ave., Colorado Springs, Colo. (1, 1925)

Boyd, Eldon M., M.A., M.D., C.M. Queen's University, Kingston, Ontario, Canada. *Professor and Head of the Department of Pharmacology.* (3, 1941)

Boyd, T. E., Ph.D. 706 S. Lincoln St., Chicago, Ill. *Professor of Physiology, Loyola University, School of Medicine.* (1, 1924)

Boyd, William C., A.M., Ph.D. Boston University School of Medicine, 80 E. Concord St., Boston, Mass. *Associate Professor of Biochemistry.* (2, 1940; 6, 1933)

Boyden, Edward A., A.M., Ph.D. University of Minnesota, Minneapolis. *Professor of Anatomy.* (1, 1929)

Boyle, Paul E., D.M.D. 25 Shattuck St., Boston, Mass. *Assistant Professor of Oral Pathology and Clinical Dentistry, Harvard School of Dental Medicine.* (4, 1939)

Bozler, Emil, Ph.D. Ohio State University, Columbus. *Assistant Professor of Physiology.* (1, 1932)

Bradbury, James T., M.S., Sc.D. Bureau of Dairy Industry, U. S. Department of Agriculture, Beltsville, Md. *Endocrinologist.* (1, 1941)

Bradley, Harold C., Ph.D. Memorial Institute Bldg., Madison, Wis. *Professor of Physiological Chemistry, University of Wisconsin.* (1, 1911; 2, 1908)

Bradley, William B., Ph.D. 3646 Lafayette Ave., Omaha, Neb. (1, 1939)

Branch, Charles F., M.D. Boston University School of Medicine, 80 E. Concord St., Boston, Mass. *Professor of Pathology.* (4, 1940)

Branch, E. Arnold G., M.D. Bureau of Laboratories, General Hospital, St. John, N. B. *Director.* (4, 1929)

Brand, Erwin, Ph.D. 630 W. 168th St., New York City. *Associate Professor of Biological Chemistry, Columbia University.* (2, 1929)

Brandes, W. W., M.D. Roosevelt Hospital, W. 59th St., New York City. (4, 1931)

Branham, Sara E., Ph.D., M.D., Sc.D. National Institute of Health, 25th and E Sts., N.W., Washington, D. C. *Senior Bacteriologist.* (6, 1926)

Branion, Hugh Douglas, M.A., Ph.D. 50 James St., Guelph, Canada. (5, 1933)

Brassfield, Charles R., Ph.D. University of Michigan, Ann Arbor. *Assistant Professor of Physiology.* (1, 1937)

Bratton, Andrew Calvin, Jr., M.A., Ph.D. Johns Hopkins School of Medicine, Baltimore, Md. *Associate in Pharmacology.* (3, 1941)

Braun, Herbert A., Ph.D. Food & Drug Administration, Federal Security Agency, Washington, D. C. *Associate Pharmacologist.* (3, 1941)

Brewer, George, M.D. University of Pennsylvania, School of Medicine, Philadelphia. *Assistant Professor of Physiology.* (1, 1937)

Bridge, Edward M., M.D. Johns Hopkins Hospital, Baltimore, Md. *Associate in Pediatrics, Johns Hopkins University.* (2, 1940)

Briggs, A. P., M.D. University of Georgia, Augusta. *Associate Professor in Biochemistry and Medicine.* (2, 1923)

Brink, Frank, Jr., Ph.D. Johnson Research Foundation, University of Pennsylvania, Philadelphia. *Fellow in Medical Physics, Johnson Research Foundation; Lecturer in Biophysics, Graduate School, University of Pennsylvania.* (1, 1942)

Brinkhous, K. M., M.D. State University of Iowa, Department of Pathology, Medical Laboratories Building, Iowa City. *Assistant Professor of Pathology.* (4, 1939)

Britton, Sydney W., M.D. University of Virginia School of Medicine, University. *Professor of Physiology.* (1, 1925)

Brodie, Bernard B., Ph.D. New York University Research Service, Welfare Hospital, Welfare Island, New York City. *Research Assistant in Biochemistry and Instructor in Medicine.* (2, 1940)

Brody, Samuel, M.A., Ph.D. Dairy Building, University of Missouri, Columbia. *Associate Professor, College of Agriculture and Agricultural Experiment Station.* (2, 1929; 5, 1933)

Bronfenbrenner, J. J., Ph.D., D.P.H. Washington University School of Medicine, St. Louis, Mo. *Professor of Bacteriology and Immunology.* (4, 1940; 6, 1918)

Bronk, Detlev W., M.S., Ph.D., Sc.D. The Eldridge Reeves Johnson Foundation for Medical Physics, University of Pennsylvania, Philadelphia. *Johnson Professor of Biophysics and Director, Johnson Foundation; Member National Academy of Sciences.* (1, 1927)

Brookes, Margaret C. Hessler, A.M., Ph.D. University of Chicago, Chicago, Ill. *Assistant Professor, Department of Home Economics.* (5, 1935)

Brooks, Chandler McCuskey, M.A., Ph.D. Johns Hopkins University School of Medicine, Baltimore, Md. *Associate Professor of Physiology.* (1, 1934)

Brooks, Clyde, Ph.D., M.D., LL.D. Louisiana State Univ. Medical Center, New Orleans. *Professor of Physiology and Pharmacology.* (1, 1910; 3, 1912)

Brooks, Matilda Moldenhauer, M.S., Ph.D. Department of Zoology, University of Cali-

fornia, Berkeley. *Research Associate in Biology.* (1, 1923)

Brooks, Sumner Cushing, Ph.D. University of California, Berkeley. *Professor of Zoology.* (1, 1923)

Broun, Goronwy Owen, M.D. 1325 S. Grand Blvd., St. Louis, Mo. *Professor of Internal Medicine, St. Louis University.* (4, 1927)

Brown, Aaron, M.D. 39 West 55th St., New York City. *Assistant Clinical Professor of Medicine, New York University College of Medicine.* (6, 1923)

Brown, Claude P., M.D. 1930 Chestnut St., Philadelphia, Pa. *Assistant Director, Pennsylvania State Board of Health Laboratories.* (6, 1913)

Brown, Dugald E. S., M.A., Ph.D. New York University College of Dentistry, 209 E. 23rd St., New York City. *Professor of Physiology.* (1, 1932)

Brown, Edgar D., Pharm.D., M.D. Paynesville, Minn. *Associate Professor of Pharmacology Emeritus.* (1, 1907; 3, 1909)

Brown, Frank A., Jr., M.A., Ph.D. Zoological Laboratories, Northwestern University, Evanston, Ill. *Associate Professor of Zoology.* (1, 1940)

Brown, John B., M.S., Ph.D. Ohio State University, Columbus. *Associate Professor of Physiological Chemistry.* (2, 1927; 5, 1934)

Brown, Rachel, M.S., Ph.D. 26 Buckingham Drive, Albany, N. Y. *Senior Biochemist, Division of Laboratories and Research, New York State Department of Health.* (6, 1933)

Browne, J. S. L., M.D., Ph.D., F.R.S.C. University Clinic, Royal Victoria Hospital, Montreal, Canada. *Assistant Professor of Medicine, McGill University.* (1, 1934)

Brubaker, Albert P., A.M., M.D. 3426 Powelton Ave., Philadelphia, Pa. *Emeritus Professor of Physiology and Medical Jurisprudence, Jefferson Medical College.* (1, 1890)

Brues, Austin M., M.D. 695 Huntington Ave., Boston, Mass. *Associate in Medicine, Harvard Medical School; Associate Physician, Collis P. Huntington Memorial Hospital; Instructor and Tutor in Biochemical Sciences, Harvard College.* (1, 1940)

Bruger, Maurice, M.D., C.M., M.Sc. 301 E. 20th St., New York City. *Associate Clinical Professor of Medicine, New York Post-Graduate Medical School of Columbia University; Chief, Division of Pathological Chemistry, New York Post-Graduate Hospital.* (2, 1935; 5, 1935)

Bruhn, John M., Ph.D. University of Alabama School of Medicine, University. *Associate Professor of Physiology and Pharmacology.* (1, 1939)

Brunschwig, Alexander, M.D. University of Chicago, Chicago, Ill. *Professor of Surgery.* (4, 1937)

Bryan, W. Ray, Ph.D. 5516 Johnson Ave., Bethesda, Md. *Research Fellow, National Cancer Institute.* (1, 1934; 4, 1940)

Buchanan, J. William, Ph.D. Northwestern University, Evanston, Ill. *Professor of Zoology.* (1, 1927)

Buchbinder, Leon, Ph.D. 122 Osborne Terrace, Newark, N. J. (6, 1934)

Buchbinder, William C., M.S., M.D. 104 S. Michigan Ave., Chicago, Ill. *Assistant Professor of Medicine, Northwestern University Medical School; Associate in Medicine, Michael Reese Hospital.* (1, 1940)

Buckner, G. Davis, Ph.D. Kentucky Agricultural Experiment Station, Lexington. *In Charge of Animal Nutrition.* (2, 1920)

Bucy, Paul C., M.S., M.D. 25 E. Washington St., Chicago, Ill. *Associate Professor of Neurology and Neurological Surgery, University of Illinois.* (1, 1933)

Buddingh, G. John, M.D. Vanderbilt University School of Medicine, Nashville, Tenn. *Associate Professor of Bacteriology.* (4, 1940)

Buell, Mary Van Rensselaer, Ph.D. Johns Hopkins Hospital, Baltimore, Md. *Associate in Medicine.* (2, 1921)

Bugbee, Edwin P., M.D. Lankenau Hospital, Philadelphia, Pa. *Assistant Roentgenologist.* (1, 1928)

Bugher, John C., M.D. Apartado 2508, Bogota, Columbia, South America. (4, 1935)

Bulatao, Emilio, M.D. University of the Philippines, Manila, P.I. *Professor of Physiology.* (1, 1924)

Bulger, Harold A., Ph.D., M.D. Barnes Hospital, 600 S. Kingshighway, St. Louis, Mo. *Assistant Professor of Medicine, Washington University.* (5, 1933)

Bull, Henry B., Ph.D. Northwestern University Medical School, 303 E. Chicago Ave., Chicago, Ill. *Associate Professor, Department of Chemistry.* (2, 1937)

Bullowa, Jesse G. M., M.D. 62 W. 87th St., New York City. *Clinical Professor of Medicine, New York University College of Medicine; Visiting Physician, Harlem Hospital; Visiting Physician, Willard Parker Hospital.* (3, 1942; 6, 1937)

Bunney, William E., Ph.D. E. R. Squibb & Sons, New Brunswick, N. J. *Director of Biologic Products Production.* (6, 1931)

Bunting, Charles H., M.D. Service Memorial Institute, Madison, Wis. *Professor of Pathology, University of Wisconsin.* (4, prior to 1920)

Bunzell, H. H., Ph.D. Box 44, General Post Office, New York City. *Director, Bunzell Laboratories.* (**2,** 1908)

Burchell, Howard B., M.D., Ph.D. Mayo Clinic, Rochester, Minn. *Instructor in Medicine, Mayo Foundation, Graduate School, University of Minnesota; Consultant in Medicine, Mayo Clinic, Rochester, Minn.* (**1,** 1942)

Burdick, H. O., M.A., Sc.D. (hon.). Alfred University, Alfred, N. Y. *Professor of Biology.* (**1,** 1940)

Burdon, Kenneth L., Sc.M., Ph.D. Louisiana State University Medical Center, New Orleans. *Assistant Professor of Immunology and Bacteriology; Consultant, United States Public Health Service.* (**6,** 1936)

Burge, W. E., A.M., Ph.D. University of Illinois, Urbana. *Associate Professor of Physiology.* (**1,** 1911)

Burk, Dean, Ph.D. National Cancer Institute, U. S. Public Health Service, Bethesda, Md. *Senior Chemist.* (**2,** 1939)

Burky, Earl L., M.S., M.D. Johns Hopkins Hospital, Baltimore, Md. *Associate Professor of Ophthalmology, Wilmer Institute of Ophthalmology, Johns Hopkins University.* (**6,** 1931)

Burnett, Theo. C., M.D. R.F.D. 1, Box 63, Carmel, Calif. *Associate Professor of Physiology Emeritus, University of California.* (**1,** 1911)

Burns, Edward L., M.D. Louisiana State University, School of Medicine, New Orleans. *Associate Professor of Pathology and Bacteriology.* (**4,** 1939)

Burr, George O., M.A., Ph.D., LL.D. University of Minnesota, Minneapolis. *Director Division of Physiological Chemistry.* (**2,** 1928; **5,** 1933)

Burrows, Montrose T., M.D. 201 N. El Molino Ave., Pasadena, Calif. (**4,** prior to 1920)

Burton, Alan C., Ph.D. Banting Institute, Toronto, Canada. (**1,** 1937)

Burton-Opitz, Russell, M.S., M.D., Ph.D. 218 Bridle Way, Palisade, N. J. *Attending Cardiologist, Lenox Hill Hospital; Attending Physician, Cumberland Hospital; Consulting Cardiologist, Engelwood, North Hudson, Holy Name and Hackensack Hospitals.* (**1,** 1902; **2,** 1906; **3,** 1919)

Bush, Milton T., Ph.D. Vanderbilt University School of Medicine, Nashville, Tenn. *Research Associate in Pharmacology.* (**3,** 1938)

Butler, Thomas C., M.D. Vanderbilt University School of Medicine, Nashville, Tenn. *Assistant Professor of Pharmacology.* (**3,** 1938)

Butt, Hugh R., M.D. U. S. Naval Hospital, Corona, Calif. (**5,** 1942)

Butts, Joseph S., M.S., Ph.D. A.P.O. 633, % Postmaster New York City. *Major, 8th Air Force Hq., Medical.* (**2,** 1936; **5,** 1936)

Cahill, William M., Ph.D. Wayne University College of Medicine, Detroit, Mich. *Assistant Professor of Physiological Chemistry.* (**2,** 1940)

Cajori, Florian A. Ph.D. 407 N. Wayne Ave., Wayne, Pa. *Major, Sanitary Corps, U. S. Army; Assistant Professor of Physiological Chemistry, University of Pennsylvania Medical School.* (**2,** 1922; **5,** 1933)

Caldwell, Mary L., A.M., Ph.D. Havemeyer Hall, Columbia University, New York City. *Assistant Professor of Chemistry.* (**2,** 1924; **5,** 1933)

Calvery, Herbert O., M.S., Ph.D. Food and Drug Administration, Federal Security Agency, Washington, D. C. *Chief, Division of Pharmacology.* (**2,** 1928; **3,** 1939)

Calvin, D. Bailey, M.A., Ph.D. School of Medicine, University of Texas, Galveston. *Associate Professor of Biological Chemistry.* (**1,** 1934; **2,** 1939)

Cameron, A. T., M.A., D.Sc., F.I.C., F.R.S.C. Medical College, Winnipeg, Manitoba, Canada. *Professor of Biochemistry, Faculty of Medicine, University of Manitoba; Biochemist, Winnipeg General Hospital.* (**1,** 1914; **2,** 1914)

Camp, Walter J. R., M.D., Ph.D. 1853 Polk St., Chicago, Ill. *Professor of Pharmacology and Therapeutics, University of Illinois.* (**3,** 1926)

Campbell, Dan H., M.S., Ph.D. Department of Chemistry, California Institute of Technology, Pasadena, Calif. *Assistant Professor of Immunochemistry.* (**6,** 1938)

Campbell, H. Louise, Ph.D. 435 W. 119th St., Apt. 9-F, New York City. *Research Assistant in Food Chemistry, Columbia University.* (**5,** 1933)

Campbell, Walter Ruggles, M.A., M.D., F.R.C.P. (C), F.R.S.C. 69 Madison Ave., Toronto, Canada. *Associate in Medicine, University of Toronto; Assistant Physician, Toronto General Hospital.* (**2,** 1922)

Cannan, R. Keith, D.Sc. 477 First Ave., New York City. *Professor of Chemistry, New York University College of Medicine.* (**2,** 1931)

Cannon, Paul R., M.D., Ph.D. University of Chicago, Chicago, Ill. *Professor of Pathology.* (**4,** 1930; **6,** 1929)

Cannon, Walter B., A.M., M.D., Sc.D., LL.D. Harvard Medical School, Boston, Mass. *George Higginson Professor of Physiology, Harvard University; Member of the National Academy of Sciences.* (**1,** 1900)

Cantarow, Abraham, M.D. Jefferson Medical College, Philadelphia, Pa. *Associate Professor of Medicine; Biochemist to the Jefferson Hospital.* (**1,** 1932; **3,** 1935)

Canzanelli, Attilio, M.D. Tufts College Medical School, 416 Huntington Ave., Boston, Mass.

Associate Professor in the Department of Physiology. (1, 1934)

Carlson, A. J., A.M., Ph.D., M.D., LL.D. Hull Physiological Laboratory, University of Chicago, Chicago, Ill. *Professor of Physiology Emeritus; Member of the National Academy of Sciences.* (1, 1904; 5, 1933)

Carmichael, Emmett B., Ph.D. School of Medicine, University of Alabama, University. *Professor of Physiological Chemistry.* (1, 1931)

Carmichael, Leonard, Ph.D., Sc.D., Litt.D., LL.D. Tufts College, Medford, Mass. *Director, the Tufts College Research Laboratory of Sensory Psychology and Physiology and President of the College.* (1, 1937)

Carpenter, Thorne M., Ph.D. 29 Vila St., Boston, Mass. *Acting Director, Nutrition Laboratory of the Carnegie Institution of Washington.* (1, 1915; 2, 1909; 5, 1933)

Carr, C. Jelleff, Ph.D. School of Medicine, University of Maryland, Baltimore. *Associate Professor of Pharmacology.* (3, 1940)

Carr, Jesse L., M.D. University of California Medical School, Third and Parnassus Aves., San Francisco. *Assistant Professor of Pathology.* (4, 1940)

Carrel, Alexis, M.D., Sc.D., LL.D. The Rockefeller Institute for Medical Research, 66th St. and York Ave., New York City. *Member Emeritus.* (1, 1906; 4, 1924)

Carter, Herbert E., Ph.D. University of Illinois, Department of Chemistry, Urbana. *Assistant Professor of Biochemistry.* (2, 1937; 5, 1941)

Cartland, George F., M.S., Ph.D. The Upjohn Co., Research Department, Kalamazoo, Mich. *Biochemist and Pharmacologist.* (2, 1936)

Cary, Charles A., S.B. Dairy Research Laboratory, Beltsville, Md. *Chief, Division of Nutrition and Physiology, Bureau of Dairy Industry, U. S. Department of Agriculture.* (2, 1920)

Casey, Albert Eugene, M.D. Jefferson and Baptist Hospitals, Birmingham, Ala. *Pathologist.* (4, 1933)

Cash, James Robert, M.D. University of Virginia, University. *Professor of Pathology.* (4, 1924)

Castle, Edward S., M.A., Ph.D. Biological Laboratories, Harvard University, Divinity Ave., Cambridge, Mass. *Assistant Professor of General Physiology.* (1, 1934)

Castle, William B., M.D., S.M. (Hon. Yale), M.D. (Hon. Utrecht). Boston City Hospital, Boston, Mass. *Professor of Medicine, Harvard Medical School; Associate Director, Thorndike Memorial Laboratory and Director, II and IV Medical Services (Harvard), Boston City Hospital.* (4, 1942)

Catchpole, Hubert Ralph, Ph.D. 333 Cedar St., New Haven, Conn. *Research Assistant in Physiology (Assistant Professor), Yale University.* (1, 1941)

Cathcart, E. P., M.D., D.Sc., LL.D. University of Glasgow, Glasgow, Scotland. *Dean of University.* (5, 1935)

Catron, Lloyd, M.D. The City Hospital, Akron, O. *Pathologist.* (4, 1939)

Cattell, J. McKeen, Ph.D., D.Sc., LL.D. Garrison-on-Hudson, N. Y. *Editor of Science, The Scientific Monthly, The American Naturalist, and School and Society; Member of the National Academy of Sciences.* (1, 1895)

Cattell, McKeen, A.M., Ph.D., M.D. Cornell University Medical College, 1300 York Ave., New York City. *Associate Professor of Pharmacology.* (1, 1923; 3, 1924)

Cerecedo, Leopold R., Ph.D. Fordham University, New York City. *Professor of Biochemistry.* (2, 1931)

Chaikoff, I. L., A.M., Ph.D., M.D. University of California, Berkeley. *Assistant Professor of Physiology.* (1, 1932)

Chalkley, Harold W., A.M., Ph.D. U. S. Public Health Service, National Institute of Health, Bethesda, Md. *Physiologist.* (1, 1932)

Chambers, Leslie Addison, M.S., Ph.D. Johnson Foundation for Medical Physics, University of Pennsylvania, Philadelphia. *Lecturer in Biophysics; Associate in Medical Physics; Associate in Pediatrics.* (1, 1940)

Chambers, Robert, A.M., Ph.D. New York University, Washington Square East, New York City. *Research Professor of Biology.* (1, 1932)

Chambers, William H., M.S., Ph.D. Cornell University Medical College, 1300 York Ave., New York City. *Associate Professor of Physiology.* (1, 1924; 5, 1933)

Chandler, Caroline A., M.D. Department of Pediatrics, Johns Hopkins Hospital, Baltimore, Md. *Instructor in Preventive Medicine.* (6, 1938)

Chanutin, Alfred, Ph.D. Box 1038, University of Virginia, University. *Professor of Biochemistry.* (2, 1925)

Chapman, C. W., M.Sc., Ph.D. University of Maryland, Baltimore. *Professor of Pharmacology.* (3, 1932)

Chargaff, Erwin, Ph.D. Columbia University, College of Physicians and Surgeons, 630 W. 168th St., New York City. *Assistant Professor of Biological Chemistry.* (2, 1935)

Charipper, Harry Adolph, M.S., Ph.D. Washington Square College of Arts and Sciences, 100 Washington Square East, New York City. *Professor of Biology and Chairman of the Department.* (1, 1941)

Chase, Aurin M., A.M., Ph.D. Department of Biology, Princeton University, Princeton, N. J. *Research Associate.* (1, 1939)

Chase, Merrill W., M.S., Ph.D. Rockefeller Institute, 66th St. and York Ave., New York City. *Member of Staff.* (**6,** 1938)

Chasis, Herbert, M.D., Med. Sc.D. 44 E. 67th St., New York City. *Assistant Professor of Medicine, New York University, College of Medicine.* (**1,** 1941)

Chatfield, Charlotte, B.S. U. S. Dept. of Agriculture, Washington, D. C. *In Charge, Food Composition Section, Bureau of Home Economics.* (**5,** 1941)

Chen, K. K., Ph.D., M.D. Eli Lilly and Co., Indianapolis, Ind. *Director of Pharmacological Research, Lilly Research Laboratories; Professor of Pharmacology, Indiana University School of Medicine, Indianapolis.* (**1,** 1929; **3,** 1942)

Cheney, Ralph H., A.M., M.S., Sc.D. Long Island University, 300 Pearl St., Brooklyn, N. Y. *Chairman, Biology Department.* (**3,** 1934)

Chesney, Alan M., M.D. The Johns Hopkins Hospital, Baltimore, Md. *Dean, Johns Hopkins Medical School; Associate Professor of Medicine.* (**4,** 1925)

Child, Charles Manning, Ph.D., D.Sc. (hon.). Jordon Hall, Stanford University, Calif. *Member, National Academy of Sciences; Professor Emeritus, University of Chicago.* (**1,** 1923)

Chittenden, Russell H., Ph.D., LL.D., Sc.D., M.D. 83 Trumbull St., New Haven, Conn. *Professor of Physiological Chemistry Emeritus, Yale University; Member, National Academy of Sciences.* (**1,** 1887; **2,** 1906; **5,** 1933)

Chow, Bacon, Ph.D. Squibb Institute for Medical Research, New Brunswick, N. J. *Associate in the Division of Pharmacology.* (**2,** 1940)

Christensen, L. Royal, Ph.D. New York University College of Medicine, 477 First Ave., New York City. *Medical Fellow, National Research Council.* (**6,** 1942)

Christian, Henry A., M.D. 1731 Beacon St., Brookline, Mass. *Hersey Professor of the Theory and Practice of Physic, Emeritus, Harvard University; Physician-in-Chief, Emeritus, Peter Bent Brigham Hospital, Boston; Visiting Physician, Beth Israel Hospital, Boston.* (**4,** 1924)

Christman, Adam A., Ph.D. University of Michigan Medical School, Ann Arbor. *Associate Professor of Physiological Chemistry.* (**2,** 1929)

Clark, Ada R., M.A., Ph.D. College of Physicians and Surgeons, 630 W. 168th St., New York City. *Instructor in Bacteriology.* (**6,** 1936)

Clark, Byron B., M.S., Ph.D. Albany Medical College, Albany, N. Y. *Associate Professor of Physiology and Pharmacology.* (**3,** 1940)

Clark, Earl P., M.S., Ph.D. Bureau of Entomology and Plant Quarantine, U. S. Dept. of Agriculture, Washington, D. C. *Senior Chemist.* (**2,** 1924)

Clark, Eliot R., M.D. University of Pennsylvania, Philadelphia. *Professor and Head of Department of Anatomy.* (**1,** 1919)

Clark, Ernest D., A.M., Ph.D. 826 Skinner Bldg., Seattle, Wash. *Director of the Laboratories, Northwest Branch, National Canners' Association; Manager, Association Pacific Fisheries.* (**2,** 1912)

Clark, Guy W., A.M., Ph.D. c/o Lederle Laboratories, Inc., Pearl River, N. Y. *Technical Director.* (**2,** 1922)

Clark, Janet Howell, A.M., Ph.D. Anderson Hall, University of Rochester, Rochester, N. Y. *Dean of the College for Women and Professor in the Division of Biological Sciences.* (**1,** 1922)

Clark, Paul F., Ph.D. University of Wisconsin Medical School, Madison. *Professor of Bacteriology.* (**4,** 1923; **6,** 1928)

Clark, William G., Ph.D. University of Minnesota, Minneapolis. *Assistant Professor of Zoology.* (**1,** 1942)

Clark, William Mansfield, M.A., Ph.D., D.Sc. Johns Hopkins University, Baltimore, Md. *Professor of Physiological Chemistry; Member National Academy of Sciences.* (**2,** 1920)

Clarke, Hans Thacher, D.Sc. (London), F.I.C. 630 W. 168th St., New York City. *Professor of Biological Chemistry, Columbia University, College of Physicians and Surgeons.* (**2,** 1929)

Clarke, J. Alexander, Jr., M.D. 334 S. 21st St., Philadelphia, Pa. (**6,** 1923)

Clarke, Robert W., Ph.D. Yale University School of Medicine, 333 Cedar St., New Haven, Conn. *Instructor in Physiology.* (**1,** 1936)

Clausen, Samuel Wolcott, M.D. Strong Memorial Hospital, Rochester, N. Y. *Professor of Pediatrics, School of Medicine, University of Rochester.* (**2,** 1922)

Cleghorn, Robert Allen, M.D., D.Sc. (Aberdeen). Department of Medicine, University of Toronto, Toronto, Ont., Canada. *Junior Demonstrator in Medicine; Junior Assistant Attending Physician, Toronto General Hospital.* (**1,** 1937)

Climenko, David Robert, M.D., Ph.D. Winthrop Chemical Co., 33 Riverside Ave., Rensselaer, N. Y. *Pharmacologist; Associate in Biochemistry and Instructor in Medicine, Albany Medical College.* (**1,** 1933)

Clowes, George Henry Alexander, Ph.D., D.Sc. (hon.), LL.D. (hon.). Eli Lilly & Co., Indianapolis, Ind. *Director of Research.* (**2,** 1914; **6,** 1919)

Coca, Arthur F., A.M., M.D. Pearl River, N. Y. Medical Director, Lederle Laboratories. (**6,** 1916)

Code, Charles F., M.D. Mayo Foundation, Rochester, Minn. *Assistant Professor of Physiology.* (**1,** 1939)

Coffey, Julia M., A.B. Division of Laboratories & Research, New York State Department of Health, Albany, N. Y. *Associate Bacteriologist.* (6, 1937)

Coghill, Robert D., M.S., Ph.D. Northern Regional Research Laboratory, U. S. Department of Agriculture, Peoria, Ill. *Chief, Fermentation Division.* (2, 1932)

Cohen, Barnett, M.S., Ph.D. Johns Hopkins University School of Medicine, 710 N. Washington St., Baltimore, Md. *Associate Professor of Physiological Chemistry.* (2, 1935)

Cohen, Milton B., M.D. 10616 Euclid Ave., Cleveland, O. *Director, The Asthma, Hay Fever and Allergy Foundation.* (6, 1931)

Cohen, Philip P., Ph.D., M.D. Department of Physiological Chemistry, Service Memorial Institutes, University of Wisconsin, Madison. *Research Associate.* (2, 1941)

Cohen, Sophia M., B.S. Division of Laboratories and Research, New York State Department of Health, Albany, N. Y. *Assistant Bacteriologist.* (6, 1938)

Cohn, Alfred E., M.D. 300 Central Park W., New York City. *Member, Rockefeller Institute for Medical Research.* (1, 1911; 3, 1913)

Cohn, Edwin J., Ph.D. 183 Brattle St., Cambridge, Mass. *Professor of Biological Chemistry, Harvard University.* (1, 1919; 2, 1919)

Cole, Arthur G., Ph.D. 1853 W. Polk St., Chicago, Ill. *Assistant Professor of Physiological Chemistry, University of Illinois College of Medicine.* (2, 1939)

Cole, Harold N., M.D. 1352 Hanna Bldg., Cleveland, O. *Clinical Professor of Dermatology and Syphilology, Western Reserve University.* (3, 1925)

Cole, Kenneth S., Ph.D. 630 W. 168th St., New York City. *Associate Professor of Physiology, College of Physicians and Surgeons, Columbia University.* (1, 1934)

Cole, Rufus, M.D., D.Sc. Mount Kisco, N. Y. *Member Emeritus, Rockefeller Institute for Medical Research.* (4, 1924; 6, 1917)

Cole, Versa V., Ph.D., M.D. Woman's Medical College, Henry Ave., & Abbottsford Rd., Philadelphia, Pa. *Associate Professor of Pharmacology.* (3, 1941)

Collett, Mary Elizabeth, A.M., Ph.D. Mather College, Western Reserve University, Cleveland, O. *Associate Professor of Biology.* (1, 1921)

Collins, Dean A., M.A., Ph.D., M.D. Temple University School of Medicine, Philadelphia, Pa. *Associate Professor of Physiology.* (1, 1938)

Collins, Russell J., A.M., M.D., F.R.C.P. (Can.) M.R.C.P. (Edin.) F.A.C.P. St. John, New Brunswick, Canada. *Medical Superintendent of St. John Tuberculosis Hospital.* (3, 1915)

Collip, J. B., A.M., Ph.D., D.Sc., M.D. McGill University, Montreal, Quebec, Canada. *Director of Research Institute of Endocrinology.* (1, 1920; 2, 1920)

Coman, Dale R., M.D. McManes Laboratory of Pathology, University of Pennsylvania School of Medicine, Philadelphia. *Assistant Professor of Pathology.* (4, 1939)

Comroe, Julius H., Jr., M.D. University of Pennsylvania Medical School, Philadelphia. *Instructor in Pharmacology.* (3, 1939)

Conant, James B., Ph.D. 5 University Hall, Cambridge, Mass. *President, Harvard University; Member National Academy of Sciences.* (2, 1932)

Concepcion, Isabelo, M.D. College of Medicine and Surgery, Manila, P.I. *Professor of Physiology, University of the Philippines.* (1, 1919)

Conklin, Ruth E., M.S., Ph.D. Vassar College. Poughkeepsie, N. Y. *Associate Professor of Physiology.* (1, 1940)

Conn, Jerome W., M.D. University Hospital, Ann Arbor, Mich. *Assistant Professor of Internal Medicine and Research Associate in Nutrition.* (5, 1942)

Cook, Donald Hunter, Ph.D. School of Tropical Medicine of Columbia University, San Juan, Puerto Rico. *Associate Professor of Chemistry.* (2, 1929)

Cooke, Robert A., A.M., Sc.D. (hon.), M.D. 60 E. 58th St., New York City. *Director, Department of Allergy, Roosevelt Hospital.* (6, 1920)

Cooley, Thomas B., M.S., M.D. 7840 Van Dyke Pl., Detroit, Mich. *Chairman of Staff, Children's Hospital of Michigan, Detroit.* (5, 1935)

Coolidge, Thomas B., M.D., Ph.D. Duke Hospital, Durham, N. C. *Assistant Professor of Biochemistry, Duke University Medical School.* (2, 1942)

Coombs, Helen C., A.M., Ph.D. Department of Hygiene, Brooklyn College, Bedford Ave. and Ave. H, Brooklyn, N. Y. *Associate Professor of Physiology and Biochemistry.* (1, 1921; 3, 1939)

Coon, Julius M., Ph.D. University of Chicago, Chicago, Ill. *Instructor in Pharmacology.* (3, 1941)

Coons, Callie Mae, Ph.D. 1200 W. 78th St., Los Angeles, Calif. (5, 1933)

Cope, Otis M., M.D. New York Medical College, Flower and Fifth Avenue Hospitals, No. 1, E. 105th St., New York City. *Professor of Physiology and Biochemistry.* (1, 1929)

Corbin, Kendall B., M.D. University of Tennessee College of Medicine, 875 Monroe, Memphis. *Professor of Anatomy.* (1, 1941)

Corcoran, Arthur Curtis, C.M., M.D. Lilly Laboratory for Clinical Research, Indianapolis City Hospital, Indianapolis, Ind. *Member of Staff.* (1, 1940)

Corey, Edward Lyman, Ph.D. School of Medicine, University of Virginia, University. *Assistant Professor of Physiology.* (1, 1931)

Cori, Carl F., M.D. Washington University School of Medicine, Kingshighway and Euclid Ave., St. Louis, Mo. *Professor of Pharmacology and Biochemistry; Member National Academy of Sciences.* (2, 1925; 3, 1934)

Cori, Gerty T., M.D. Washington University School of Medicine, St. Louis, Mo. *Research Associate in Pharmacology and Biochemistry.* (2, 1927; 3, 1934)

Corley, Ralph Conner, Ph.D. Department of Chemistry, Purdue University, Lafayette, Ind. *Professor of Biochemistry.* (2, 1927)

Corper, Harry J., M.D., Ph.D. 1295 Clermont St. Denver, Colo. *Director of Research, National Jewish Hospital.* (2, 1912)

Corwin, Warren C., M.D. Captain, M.C. AUS, Army Flying School, Greenville, Miss. (4, 1940)

Co Tui, Frank, M.D. New York University College of Medicine, 477 First Ave., New York City. *Associate Professor of Experimental Surgery.* (3, 1931)

Cowgill, George Raymond, Ph.D. 333 Cedar St., New Haven, Conn. *Associate Professor of Physiological Chemistry, Yale University.* (1, 1923; 2, 1922; 5, 1933)

Cox, Gerald J., M.S., Ph.D. 3803 S St., N.W., Washington, D. C. (2, 1930; 5, 1935)

Cox, Warren M., Jr., Ph.D. Mead Johnson & Co., Evansville, Ind. *Research Biochemist.* (2, 1935)

Craig, L. C., M.S., Ph.D. Rockefeller Institute, 66th St. and York Ave., New York City. *Associate in Chemical Pharmacology.* (2, 1938)

Crampton, E. W., Ph.D. Macdonald College, Quebec, Canada. *Associate Professor of Animal Nutrition.* (5, 1940)

Crandall, Lathan A., Jr., M.D., Ph.D. University of Tennessee College of Medicine, Memphis. *Professor of Physiology.* (1, 1930; 5, 1940)

Cretcher, Leonard H., Ph.D. Mellon Institute of Industrial Research, University of Pittsburgh, Pittsburgh, Pa. *Assistant Director and Head of the Department of Research in Pure Chemistry.* (2, 1930)

Crider, Joseph O., M.D. Jefferson Medical College, Philadelphia, Pa. *Associate Professor of Physiology and Assistant Dean.* (1, 1935)

Crile, George W., A.M., M.D., F.R.C.S. Cleveland Clinic, 2020 E. 93rd St., Cleveland, O. *Professor Emeritus of Surgery, Western Reserve University School of Medicine; Director, Cleveland Clinic Foundation; Surgeon, Cleveland Clinic Hospital.* (1, 1907; 3, 1909)

Crisler, George R., Ph.D., M.D. Patrician Hotel, Miami Beach, Fla. (1, 1930)

Crittenden, Phoebe J., M.S., Ph.D. 106 W. Underwood St., Chevy Chase, Md. *Instructor in Pharmacology, George Washington University Medical School.* (1, 1937; 3, 1937)

Cromwell, Hobart W., Sc.D. Abbott Laboratories, North Chicago, Ill. *Bacteriologist.* (6, 1929)

Crozier, William J., Ph.D. Biological Laboratories, Harvard University, Cambridge, Mass. *Professor of General Physiology.* (1, 1928)

Cruickschank, Ernest W. H., M.D., D.Sc., Ph.D. M.R.C.P., F.R.S.E. Marischal College, University of Aberdeen, Aberdeen, Scotland. *Professor of Physiology.* (1, 1931)

Csonka, F. A., Ph.D. Protein and Nutrition Research Division, Bureau of Agricultural Chemistry and Eng., U. S., Department of Agriculture, Washington, D. C. *Senior Chemist.* (2, 1924)

Culler, Elmer A. K., Ph.D. University of Rochester, Rochester, N. Y. *Professor of Psychology and Director of the Laboratory.* (1, 1936)

Cunningham, Raymond W., M.S., Ph.D. Temple University School of Medicine, Philadelphia, Pa. *Instructor and Research Fellow in Pharmacology.* (3, 1941)

Cunningham, Robert Sydney, A.M., M.D., Sc.D. Albany Medical College, Albany, N. Y. *Professor of Anatomy and Dean.* (1, 1923)

Curnen, Edward C., M.D. Hospital of Rockefeller Institute, 66th St. and York Ave., New York City. *Assistant Resident Physician, Hospital of The Rockefeller Institute; Assistant, Rockefeller Institute; Lieut. (j.g.) M.C. V(S) U.S.N.R. on active duty.* (6, 1941)

Curtis, George Morris, M.A., Ph.D., M.D. Kinsman Hall, Ohio State University, Columbus. *Professor of Surgery; Chairman, Department of Research Surgery.* (1, 1933; 4, 1933)

Curtis, Howard J., M.A., Ph.D. College of Physicians and Surgeons, 630 W. 168th St., New York City. *Assistant Professor of Physiology.* (1, 1940)

Cutler, Elliott C., M.D. Peter Bent Brigham Hospital, Boston, Mass. *Moseley Professor of Surgery, Harvard Medical School; Surgeon-in-Chief, Peter Bent Brigham Hospital.* (4, 1927)

Cutting, Reginald A., M.D., Ph.D. Georgetown University School of Medicine, 3900 Reservoir Road, N.W., Washington, D. C. *Professor of Physiology and Director of the Department.* (1, 1939)

Cutting, Windsor C., M.D. Stanford University School of Medicine, San Francisco, Calif. *Assistant Professor of Therapeutics.* (3, 1939)

Daft, Floyd Shelton, Ph.D. National Institute of Health, Washington, D. C. *Senior Biochemist.* (5, 1941)

Daggs, Ray Gilbert, Ph.D. 311 Cloverleaf Ave., San Antonio, Texas. *Major, San. Corps; Associate Professor of Physiology, University of Vermont, College of Medicine, Burlington (on leave).* (1, 1935; 5, 1933)

Dakin, Henry D., D.Sc., LL.D., Ph.D., F.I.C., F.R.S. Scarborough-on-Hudson, N. Y. (2, 1906)

Dalton, Albert J., M.A., Ph.D. National Institute of Health, Bethesda, Md. *Cytologist.* (4, 1942)

D'Amour, Fred E., M.S., Ph.D. 2311 S. Josephine St., Denver, Colo. *Associate Professor, Department of Zoology, University of Denver.* (1, 1934)

D'Amour, Marie C., Ph.D., M.D. Tulane University, School of Medicine, New Orleans, La. *Instructor in Pharmacology.* (1, 1934)

Daniels, Amy L., Ph.D. College Highway, R.F.D. Avon, Conn. (2, 1919; 5, 1933)

Danielson, Irvin S., Ph.D. Pearl River Apartments, Apt. 3H, Pearl River, N. Y. *Research Chemist.* (2, 1937)

Dann, W. J., Ph.D. Box 3205, Duke Hospital, Durham, N. C. *Associate Professor of Physiology.* (2, 1937; 5, 1938)

Darrow, Chester W., Ph.D. Institute for Juvenile Research, 907 S. Wolcott St., Chicago, Ill. *Research Psychologist, Institute for Juvenile Research; Associate in Physiology, University of Illinois College of Medicine.* (1, 1937)

Darrow, Daniel Cady, M.D. New Haven Hospital, New Haven, Conn. *Associate Professor of Pediatrics, Yale University.* (2, 1936)

Davenport, Horace Willard, B.Sc. (Oxon), Ph.D. University of Pennsylvania, School of Medicine, Philadelphia. *Instructor in Physiology.* (1, 1942)

David, Norman Austin, M.D. University of Oregon Medical School, Portland. *Professor of Pharmacology.* (3, 1934)

Davidsohn, Israel, M.D. Mount Sinai Hospital, 2750 W. 15th Place, Chicago, Ill. *Pathologist and Director of Laboratories, Mt. Sinai Hospital; Assistant Professor of Pathology, College of Medicine, University of Illinois.* (4, 1939; 6, 1929)

Davis, Hallowell, M.D. Harvard Medical School, Boston, Mass. *Associate Professor of Physiology.* (1, 1925)

Davis, John Emerson, M.S., Ph.D. Dept. of Physiology and Pharmacology, Univ. of Arkansas School of Medicine, Little Rock. *Associate Professor of Pharmacology.* (1, 1941; 3, 1941)

Davson, Hugh, M.Sc., D.Sc. Dalhousie University, Halifax, N.S., Canada. *Associate Professor of Physiology.* (1, 1941)

Dawson, James Robertson, Jr., M.D. Vanderbilt Medical School, Nashville, Tenn. *Associate Professor.* (4, 1940)

Dawson, Martin H., M.D., C.M. Presbyterian Hospital, 620 W. 168th St., New York City. *Associate Professor of Medicine.* (4, 1934; 6, 1934)

Dawson, Percy M., M.D. 806 W. 31st St., Austin, Texas. (1, 1900)

Day, Harry G., D.Sc. University of Indiana, Bloomington. *Associate Professor, Dept. of Chemistry.* (5, 1940)

Day, Paul L., M.A., Ph.D. University of Arkansas School of Medicine, Little Rock. *Professor of Physiological Chemistry.* (2, 1934; 5, 1933)

DeEds, Floyd, M.A., Ph.D. Dept. of Pharmacology, Stanford University School of Medicine, San Francisco, Calif. *Principal Pharmacologist, U. S. Department of Agriculture, Bureau of Agricultural Chemistry and Engineering.* (2, 1937; 3, 1927)

Defandorf, James Holmes, Ph.D. Assistant Chief, Medical Research Division, Edgewood Arsenal, Md. *Senior Pharmacologist.* (3, 1940)

de Gara, Paul F., M.D. 200 Pinehurst Ave., New York City. *Instructor in Pathology, Cornell University Medical College.* (6, 1941)

DeGraff, Arthur C., M.D. New York University College of Medicine, New York City. *Professor of Theraepeutics.* (3, 1937)

Deichmann, Wilhelm, M.Sc., Ph.D. 527 McAlpin, Cincinnati, O. *Instructor, Kettering Laboratory of Applied Physiology; Instructor in Physiology, University of Cincinnati, College of Medicine.* (3, 1941)

Dempsey, Edward W., Sc.M., Ph.D. Harvard Medical School, Boston, Mass. *Instructor in Physiology.* (1, 1940)

Derbyshire, Arthur J., Ph.D. Wayne University College of Medicine, Detroit, Mich. *Assistant Professor of Anatomy.* (1, 1939)

de Savitsch, Eugene, M.D. Suite 24, 1150 Connecticut Ave., Washington, D. C. *Consulting Surgeon, Glen Dale Sanatorium.* (4, 1934)

Deuel, Harry J., Jr., Ph.D. University of Southern California Medical School, Los Angeles. *Professor of Biochemistry.* (1, 1928; 2, 1924; 5, 1933)

Deulofeu, Venancio, D. Chem. Parera 77, Buenos Aires, Argentina. *Professor of Organic Chemistry, University of Buenos Aires.* (2, 1942)

Dienes, Louis, M.D. Massachusetts General Hospital, Boston. *Bacteriologist.* (6, 1924)

Dill, David Bruce, M.A., Ph.D. Aeromedical Research Laboratory, Wright Field, Dayton, O. *Lt. Colonel, Air Corps, U. S. Army; Professor of Industrial Physiology, Harvard University, on leave.* (1, 1941; 2, 1927; 5, 1936)

Dille, James M., M.S., Ph.D. College of Pharmacy, University of Washington, Seattle. *Associate Professor of Pharmacology.* (3, 1939)

Dillon, Robert T., M.S., Ph.D. % G. D. Searle and Co., Box 5110, Chicago, Ill. *Head, Analytical Division.* (2, 1934)

Dingle, John H., Sc.D., M.D. Thorndike Memorial Laboratory, Boston City Hospital, Boston, Mass. *Instructor and Francis Weld Peabody Fellow in Medicine, and Instructor in Bacteriology and Immunology, Harvard Medical School; Assistant Physician, Thorndike Memorial Laboratory; Assistant in Medicine, Boston City Hospital; Consultant to Secretary of War in Infectious Diseases.* (6, 1941)

Dixon, Harold M., M.D. University of Pennsylvania, Philadelphia. *Associate in Pathology; Chief of the Division of Pathology, Philadelphia General Hospital.* (4, 1936)

Doan, Charles A., M.D. Ohio State University, College of Medicine, Columbus. *Professor and Chairman of the Department of Medicine; Director of Medical Research.* (4, 1928)

Dochez, A. Raymond, M.D., Sc.D. (hon.). Presbyterian Hospital, 620 W. 168th St., New York City. *John E. Borne Professor of Medical and Surgical Research, Columbia University; Member of National Academy of Sciences.* (4, prior to 1920; 6, 1922)

Dohan, F. Curtis, M.D. 80 Princeton Rd., Cynwyd, Pa. *Fellow, George S. Cox Medical Research Institute; Associate in Medicine, University of Pennsylvania, Philadelphia.* (1, 1941)

Doisy, Edward A., M.S., Ph.D., Sc.D. St. Louis University School of Medicine, St. Louis, Mo. *Professor of Biological Chemistry; Member National Academy of Sciences.* (2, 1920)

Dominguez, Rafael, M.D. Saint Luke's Hospital, Cleveland, O. *Director of Laboratories, St. Luke's Hospital; Associate in Pathology, Western Reserve University.* (1, 1935)

Donahue, D. D., D.Sc. Division of Industrial Hygiene, National Institute of Health, Bethesda, Md. *Physiologist, Toxicology Section, Division of Industrial Hygiene, U. S. Public Health Service.* (3, 1941)

Dooley, M. S., M.D. 766 Irving Ave., Syracuse, N. Y. *Professor of Pharmacology, College of Medicine, Syracuse University.* (3, 1923)

Dorfman, Ralph I., Ph.D. Dept. of Biochemistry, Western Reserve University School of Medicine, Cleveland, O. *Assistant Professor of Biochemistry.* (2, 1940)

Dotti, Louis Basil, M.A., Ph.D. New York Medical College, Flower and Fifth Avenue Hospitals, 105th St., New York City. *Assistant Professor of Physiology and Biochemistry.* (1, 1937)

Doty, J. Roy, Ph.D. Louisiana State University School of Medicine, New Orleans. *Assistant Professor of Physiology.* (2, 1941)

Dow, Philip, Ph.D. University of Georgia School of Medicine, Augusta. *Associate Professor of Physiology.* (1, 1939)

Dow, Robert S., M.D., Ph.D. University of Oregon Medical School, Portland. *Assistant Professor of Anatomy.* (1, 1940)

Downs, Ardrey W., M.A., M.D., D.Sc., F.A.C.P. University of Alberta, Edmonton, Canada. *Professor of Physiology and Pharmacology.* (1, 1917)

Downs, Cora M., Ph.D. Department of Bacteriology, University of Kansas, Lawrence. *Professor of Bacteriology.* (6, 1929)

Drabkin, David L., M.D. Medical School, University of Pennsylvania, Philadelphia. *Associate Professor of Physiological Chemistry.* (2, 1928; 5, 1934)

Dragstedt, Carl A., Ph.D., M.D. Northwestern University Medical School, 303 E. Chicago Ave., Chicago, Ill. *Professor of Pharmacology.* (1, 1928; 3, 1932)

Dragstedt, Lester R., M.D., Ph.D. University of Chicago, Chicago, Ill. *Professor of Surgery.* (1, 1920)

Draize, J. H., Ph.D. Division of Pharmacology, Food & Drug Administration, U. S. Dept. of Agriculture, Washington, D. C. *Pharmacologist.* (3, 1940)

Drake, T. G. H., M.B., F.R.C.P. (c). University of Toronto, Toronto, Canada. *Junior Demonstrator in Paediatrics, Department of Medicine, University of Toronto; Clinical Assistant on Active Staff and Associate Director Research Laboratory, Hospital for Sick Children.* (5, 1936)

Draper, William B., M.Sc., M.D. University of Colorado School of Medicine, 4200 E. 9th Ave., Denver. *Associate Professor of Physiology and Pharmacology.* (3, 1938)

Dresbach, Melvin, M.S., M.D. The School of Medicine, University of Pennsylvania, Philadelphia. *Visiting Fellow in Physiology.* (1, 1912)

Dreyer, Nicholas Bernard, M.A. (Oxon). Long Island College of Medicine, 350 Henry St., Brooklyn, N. Y. *Associate Professor of Physiology and Pharmacology.* (3, 1942)

Drinker, Cecil K., M.D. Harvard University School of Public Health, Boston, Mass. *Professor of Physiology and Dean.* (1, 1915)

Drinker, Katherine R., M.D. Harvard School of Public Health, 55 Shattuck St., Boston, Mass. *Instructor in Public Health.* (1, 1915)

Drury, Douglas R., M.D. University of Southern California, Los Angeles. *Professor of Physiology.* (1, 1932)

Dubin, Harry E., Ph.D. 250 E. 43rd St., New York City. *President, H. E. Dubin Laboratories, Inc.* (2, 1925)

DuBois, Eugene F., M.D. Cornell University Medical College, 1300 York Ave., New York City. *Professor and Head of the Department of Physiology and Biophysics; Attending Physician, New York Hospital; Member, National Academy of Sciences.* (1, 1913; 3, 1921; 5, 1935)

Dubos, Rene J., Ph.D., D.Sc. Harvard Medical School, Boston, Mass. *Professor of Tropical Medicine.* (6, 1938)

Dukes, H. H., D.V.M., M.S. New York State Veterinary College, Cornell University, Ithaca, N. Y. *Professor of Veterinary Physiology.* (1, 1934)

Dulaney, Anna D., A.M., Ph.D. Pathological Institute, University of Tennessee, Memphis. *Assistant Professor of Bacteriology, Medical School.* (6, 1924)

Dumke, Paul Rudolph, M.D. Department of Pharmacology, The Medical School, University of Pennsylvania, Philadelphia. *Instructor in Pharmacology.* (3, 1942)

Dunlap, Charles E., M.D. Harvard Medical School, 25 Shattuck St., Boston, Mass. *Instructor in Pathology.* (4, 1942)

Dunn, Max Shaw, Ph.D. University of California, Los Angeles. *Associate Professor of Chemistry.* (2, 1933)

Durrant, Edwin Poe, M.A., Ph.D. Ohio State University, Columbus. *Associate Professor of Physiology.* (1, 1928)

Dutcher, R. Adams, M.S., M.A., D.Sc. Pennsylvania State College, State College. *Professor and Head of Department of Agricultural Biochemistry.* (2, 1920; 5, 1933)

Duval, Charles Warren, M.D. Tulane University, New Orleans, La. *Professor of Pathology and Bacteriology.* (4, prior to 1920)

du Vigneaud, Vincent, M.S., Ph.D. Cornell University Medical College, 1300 York Ave., New York City. *Professor of Biochemistry.* (2, 1929; 5, 1934)

Dworkin, Simon, D.D.S., M.D., C.M. Biology Building, McGill University, Montreal, Quebec, Canada. *Lecturer in Physiology, Faculty of Medicine.* (1, 1931)

Dye, J. A., Ph.D. James Law Hall, Cornell University, Ithaca, N. Y. *Associate Professor of Physiology.* (1, 1929)

Dye, Marie, M.S., Ph.D. Michigan State College, East Lansing. *Dean of Division of Home Economics.* (2, 1929; 5, 1933)

Dyer, Helen M., M.S., Ph.D. National Cancer Institute, Bethesda, Md. *Research Associate.* (2, 1936; 5, 1937)

Eadie, George S., Ph.D. Duke University School of Medicine, Box 3709, Durham, N. C. *Professor of Physiology and Pharmacology.* (1, 1929; 3, 1940)

Eagle, Harry, M.D. Johns Hopkins Hospital, Baltimore, Md. *Passed Assistant Surgeon, U. S. Public Health Service; Lecturer in Medicine, Johns Hopkins University Medical School.* (4, 1936)

Earle, Wilton R., Ph.D. U. S. Public Health Service, National Cancer Institute, Bethesda, Md. *Senior Cytologist.* (4, 1940)

Eaton, Alonzo Guy, M.A., Ph.D. Louisiana State University Medical Center, New Orleans. *Assistant Professor of Physiology.* (1, 1933)

Eaton, Monroe D., M.D. State Department of Public Health, Influenza Laboratory, 1392 University Ave., Berkeley, Calif. *Staff Member, International Health Division of The Rockefeller Foundation.* (6, 1937)

Ecker, E. E., Ph.D. Western Reserve University, Cleveland, O. *Professor of Immunology, Western Reserve University, and Visiting Bacteriologist and Immunologist to Lakeside, Babies' and Children's Hospitals and the City of Cleveland.* (4, 1925)

Eckstein, Henry C., M.S., Ph.D. 320 W. Medical Building, University of Michigan, Ann Arbor. *Associate Professor of Biological Chemistry.* (2, 1925)

Eddy, Nathan B., M.D. National Institute of Health, Bethesda, Md. *Principal Pharmacologist, United States Public Health Service.* (1, 1919; 3, 1929)

Eddy, Walter H., A.M., Ph.D. Teachers' College, Columbia University, New York City. *Professor of Physiological Chemistry.* (2, 1913; 5, 1933)

Edsall, John Tileston, M.D. Harvard Medical School, Boston, Mass. *Associate Professor of Biological Chemistry and Tutor in Biochemical Sciences.* (2, 1931).

Edwards, Dayton J., Ph.D. 1300 York Ave., New York City. *Associate Professor of Physiology; Assistant Dean, Cornell University Medical College.* (1, 1921)

Edwards, Jesse E., M.D. *Captain, M.C., Army Medical Center, Washington, D. C.* (4, 1941)

Edwards, J. Graham, A.M., Ph.D. 24 High St., Buffalo, N. Y. *Assistant Professor of Anatomy, University of Buffalo.* (1, 1932)

Eggerth, Arnold H., Ph.D. Hoagland Laboratory, 335 Henry St., Brooklyn, N. Y. *Associate Professor of Bacteriology, Long Island College of Medicine.* (4, 1925)

Ehrenstein, Maximilian R., Ph.D. 806 Maloney Clinic, University of Pennsylvania Hospital, 36th and Spruce Sts., Philadelphia. *Assistant Professor of Chemistry assigned to Medicine.* (2, 1942)

Eichelberger, Lillian, Ph.D. The Lasker Foundation for Medical Research, University of Chicago, Department of Medicine, Chicago, Ill. *Assistant Professor of Biochemistry.* (**2,** 1937)

Eisenman, Anna J., Ph.D. Yale University, New Haven, Conn. *Instructor, Department of Internal Medicine.* (**2,** 1930)

Elderfield, Robert C., Ph.D. Department of Chemistry, Columbia University, New York City. *Associate Professor.* (**2,** 1934)

Elftman, Herbert, M.A., Ph.D. College of Physicians and Surgeons, Columbia University, 630 W. 168th St., New York City. *Instructor in Anatomy.* (**1,** 1940)

Eliot, Martha M., M.D. United States Children's Bureau, Washington, D. C. *Assistant Chief.* (**5,** 1933)

Elliott, K. Allan C., M.Sc., Ph.D. Institute of the Pennsylvania Hospital, 111 N. 49th St., Philadelphia. *Assistant Professor of Biochemistry in Psychiatry, University of Pennsylvania.* (**2,** 1937)

Ellis, Frederick W., M.D. Monson, Mass. (**1,** 1887)

Ellis, Lillian N., Ph.D. Adelphi College, Garden City, N. Y. (**5,** 1940)

Ellis, Max Mapes, A.M., Ph.D., Sc.D. University of Missouri, Columbia. *Professor of Physiology and Pharmacology.* (**1,** 1923)

Ellis, N. R., M.S. Bureau of Animal Industry, U. S. Department of Agriculture, Beltsville Research Center, Beltsville, Md. *Acting Chief, Animal Nutrition Division.* (**2,** 1928; **5,** 1933)

Elser, William J., M.D. 1300 York Ave., New York City. *Emeritus Professor of Applied Pathology and Bacteriology, New York Hospital.* (**6,** 1920)

Elvehjem, Conrad Arnold, M.S., Ph.D., Sc.D. Biochemistry Building, University of Wisconsin, Madison. *Professor of Biochemistry; Member National Academy of Sciences.* (**2,** 1931; **5,** 1933)

Emerson, George A., M.S., Ph.D. West Virginia School of Medicine, Morgantown. *Associate Professor and Head, Department of Pharmacology.* (**3,** 1935)

Emerson, Gladys A., Ph.D. Merck Institute of Therapeutic Research, Rahway, N. J. *Nutritionist.* (**5,** 1942)

Emerson, Oliver H., Ph.D. Institute of Experimental Biology, University of California, Berkeley. *Research Associate.* (**2,** 1938)

Emery, Frederick E., D.V.M., M.S., Ph.D. University of Buffalo Medical School, Buffalo, N. Y. *Assistant Professor of Physiology.* (**1,** 1930)

Emmett, Arthur D., M.A., Ph.D. Research Department, Parke, Davis & Co., Detroit, Mich. *Assistant Director of Research.* (**2,** 1908; **5,** 1933)

Enders, John F., A.M., Ph.D. Department of Bacteriology, Medical School, Harvard University, Boston, Mass. *Assistant Professor of Bacteriology and Immunology.* (**6,** 1936)

Engle, Earl Theron, Ph.D. College of Physicians and Surgeons, Columbia University, 630 W. 168th St., New York City. *Professor of Anatomy.* (**1,** 1930)

Epstein, Albert A., M.D. 1111 Madison Ave., New York City. *Physician, Beth Israel Hospital; Physician, Hospital for Joint Diseases.* (**2,** 1912)

Erickson, Cyrus C., M.D. Duke University School of Medicine, Durham, N. C. *Associate in Pathology.* (**4,** 1941)

Erlanger, Joseph, M.D., LL.D., Sc.D. Washington University School of Medicine, 4580 Scott Ave., St. Louis, Mo. *Professor of Physiology; Member of the National Academy of Sciences.* (**1,** 1901)

Espe, Dwight L., Ph.D. Iowa State College, Ames. *Assistant Research Professor in Dairy Husbandry.* (**1,** 1940)

Essex, Hiram E., M.S., Ph.D. Mayo Foundation, Rochester, Minn. *Associate Professor of Physiology, Institute of Experimental Medicine.* (**1,** 1932; **3,** 1940)

Ets, Harold N., Ph.D. 706 S. Wolcott Ave., Chicago, Ill. *Associate Professor of Physiology and Pharmacology, Loyola University School of Medicine.* (**1,** 1931; **3,** 1931)

Evans, Earl Alison, Jr., Ph.D. Department of Biochemistry, University of Chicago, Chicago, Ill. *Associate Professor and Chairman of Department.* (**2,** 1939)

Evans, Everett Idris, M.D., Ph.D. Department of Surgery, Medical College of Virginia, Richmond. *Assistant Professor of Surgery; Responsible Investigator, Committee on Medical Research, National Research Council.* (**1,** 1935)

Evans, Gerald Taylor, M.D., Ph.D. University of Minnesota Hospitals, Minneapolis. *Director of Laboratory Service, University of Minnesota Hospitals; Associate Professor of Medicine, University of Minnesota.* (**1,** 1942)

Evans, Herbert M., M.D. University of California, Berkeley. *Professor of Anatomy and Director of Institute of Experimental Biology; Member of the National Academy of Sciences.* (**1,** 1919)

Evans, William E., Jr., M.S., Ph.D. University of Maryland Medical School, Baltimore. *Assistant Professor of Pharmacology.* (**3,** 1940)

Eveleth, D. F., Ph.D. University of Arkansas, Fayetteville. *Professor of Bacteriology and Veterinary Science.* (**2,** 1939)

Everett, Mark Reuben, Ph.D. University of Oklahoma Medical School, Oklahoma City. *Professor of Biochemistry.* (**2,** 1929)

Ewing, P. L., M.S., Ph.D. University of Texas School of Medicine, Galveston. *Associate in Pharmacology.* (3, 1938)

Eyster, John A. English, M.D. University of Wisconsin, Madison. *Professor of Physiology.* (1, 1906; 3, 1908)

Fahr, George, M.D. University of Minnesota Medical School, Minneapolis. *Professor of Clinical Medicine.* (1, 1913; 3, 1940)

Failey, Crawford F., Ph.D. 416 S. 6th St., Terre Haute, Ind. *Associate Professor of Pharmacology.* (2, 1933)

Fairhall, Lawrence T., M.A., Ph.D. U. S. Public Health Service, Washington, D. C. *Principal Industrial Toxicologist.* (2, 1924).

Falk, K. George, Ph.D. 40 E. 66th St., New York City. *Director, Laboratory of Industrial Hygiene.* (2, 1913)

Famulener, Lemuel W., Ph.C., M.D. Pathological Laboratory, St. Luke's Hospital, 113th St. and Amsterdam Ave., New York City. *Bacteriologist.* (6, 1920)

Farber, Sidney, M.D. Harvard Medical School, 25 Shattuck St., Boston, Mass. *Assistant Professor of Pathology.* (4, 1934)

Farmer, Chester J., A.M. Northwestern Medical School, 303 E. Chicago Ave., Chicago, Ill. *Professor of Chemistry.* (2, 1935)

Farr, Lee E., M.D. 800 Devon Ave., Los Angeles, Calif. (4, 1941)

Farrell, James I., Ph.D., M.D. 636 Church St., Evanston, Ill. (1, 1938)

Fassett, David W., M.D. Department of Therapeutics, New York University College of Medicine, 414 E. 26 St., New York City. *Fellow, Department of Therapeutics.* (3, 1942)

Fay, Marion, M.A., Ph.D. Woman's Medical College of Pennsylvania, East Falls, Philadelphia. *Professor of Physiological Chemistry.* (2, 1937)

Feldman, William H., D.V.M., M.S. Institute of Experimental Medicine, Mayo Clinic, Rochester, Minn. *Associate in the Division of Experimental Surgery and Pathology.* (4, 1934)

Fellows, Edwin J., M.S., Ph.D. Temple University School of Medicine, Philadelphia, Pa. *Instructor in Pharmacology.* (3, 1939)

Felton, Lloyd D., M.D., D.Sc. Division of Infectious Diseases, National Institute of Health, 25th and E Sts., N.W., Washington, D. C. *Senior Surgeon, United States Public Health Service.* (6, 1926)

Fenn, Wallace Osgood, A.M., Ph.D. University of Rochester School of Medicine and Dentistry, Rochester, N. Y. *Professor of Physiology.* (1, 1924)

Fenning, Con, M.D., M.A. University of Utah School of Medicine, Salt Lake City. *Associate Professor of Pharmacology and Physiology.* (1, 1942)

Ferguson, James Kenneth Wallace, M.A., M.D. University of Toronto, Toronto, Ont., Canada. *Assistant Professor of Pharmacy and Pharmacology.* (1, 1933; 3, 1941)

Ferguson, John Howard, M.D., M.A., L.M.S.S.A. University of Michigan, Ann Arbor. *Assistant Professor of Pharmacology.* (1, 1933; 3, 1939)

Ferguson, L. Kraeer, M.D. 133 S. 36th St. Philadelphia, Pa. *Assistant Professor of Surgery, University of Pennsylvania; Surgeon, Philadelphia General Hospital; Assistant Surgeon, University of Pennsylvania Hospital.* (4, 1935)

Ferry, John Douglass, Ph.D. Woods Hole Oceanographic Institution, Woods Hole, Mass. *Associate Chemist, U. S. Navy Antifouling Project.* (2, 1941)

Ferry, Newell S., M.D. Parke, Davis & Co., Detroit, Mich. *Assistant Director of Research.* (6, 1916)

Ferry, Ronald M., M.D. 966 Memorial Drive, Cambridge, Mass. *Master of John Winthrop House.* (2, 1924)

Fevold, Harry L., M.S., Ph.D. Western Regional Research Laboratory, Albany, Calif. *Senior Biochemist.* (2, 1942)

Field, John, II, A.M., Ph.D. Stanford University, Calif. *Associate Professor of Physiology.* (1, 1930)

Fincke, Margaret L., Ph.D. Oregon State College, Corvallis. *Associate Professor of Foods and Nutrition, School of Home Economics.* (5, 1940)

Findley, Thomas, Jr., M.D. Ochsner Clinic, 3503 Prytania, New Orleans, La. *Head of the Department of Internal Medicine, Ochsner Clinic, New Orleans; Assistant Professor of Clinical Medicine, Tulane University School of Medicine.* (1, 1938)

Fine, Morris S., Ph.D. Central Laboratories, General Foods Corporation, Hoboken, N. J. *Director of Research.* (2, 1912; 5, 1933)

Finland, Maxwell, B.S. Boston City Hospital, Boston, Mass. *Assistant Professor of Medicine, Harvard Medical School.* (6, 1941)

Firor, Warfield Monroe, M.D. Johns Hopkins Hospital, Baltimore, Md. *Associate Professor of Surgery, Johns Hopkins University.* (1, 1932)

Fischer, Ernst, M.D., Dr. habil. Medical College of Virginia, Richmond. *Associate Professor of Physiology and Pharmacology.* (1, 1936)

Fischer, Hermann O. L., Ph.D. Banting Institute, 100 College St., University of Toronto, Toronto, Canada. *Research Professor of Organic Chemistry.* (2, 1940)

Fischer, Martin H., M.D., hon. Pharm. D., Sc.D. University of Cincinnati College of Medicine,

Eden Ave., Cincinnati, O. *Professor of Physiology.* (1, 1901; 2, 1919)

Fishberg, Ella H., M.A., M.D. Beth Israel Hospital, Stuyvesant Park East, New York City. *Biochemist.* (2, 1931)

Fisher, Kenneth C., M.A., Ph.D. University of Toronto, Toronto, Ont., Canada. *Assistant Professor of Physiological Zoology.* (1, 1940)

Fiske, Cyrus H., M.D. Harvard Medical School, Boston, Mass. *Professor of Biological Chemistry.* (2, 1914)

Fitzgerald, Mabel P., 54 A, George Sq., Edinburgh, Scotland. (1, 1913)

Fitzhugh, O. Garth, Ph.D. Division of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D. C. *Pharmacologist.* (3, 1940)

Fleischmann, Walter, M.D., Ph.D. Harriet Lane Home, Johns Hopkins Hospital, Baltimore, Md. *Instructor in Pediatrics, Johns Hopkins University.* (1, 1940)

Fleisher, Moyer S., M.D. Jewish Hospital, St. Louis, Mo. *Research Bacteriologist.* (4, 1924; 6, 1932)

Flexner, Louis B., M.D. Department of Embryology, Carnegie Institution of Washington, Wolfe and Madison Sts., Baltimore, Md: *Research Associate.* (1, 1933)

Flock, Eunice V., Ph.D. Mayo Clinic, Rochester, Minn. *Assistant Professor of Biochemistry, University of Minnesota*; *Associate in the Division of Experimental Medicine, The Mayo Foundation.* (2, 1940)

Florman, Alfred L., M.D. Mt. Sinai Hospital, 1 E. 100th St., New York City. *Resident in Pediatrics.* (6, 1942)

Flosdorf, Earl W., Ph.D. 305 Lincoln Ave., Lansdowne, Pa. *Research—University of Pennsylvania School of Medicine.* (6, 1941)

Floyd, Cleveland, M.D., Sc.D. 246 Marlborough St., Boston, Mass. *Chief Examiner, Boston Health Dept.* (6, 1916)

Folch, Jordi, M.D. Rockefeller Institute for Medical Research, New York City. *Associate.* (2, 1941)

Follensby, Edna M., Ph.G. 80 E. Concord St., Boston, Mass. *Research Assistant, Evans Memorial.* (6, 1933)

Follis, Richard H., Jr., M.D. Johns Hopkins Hospital, Baltimore, Md. *Associate in Pathology, The Johns Hopkins University.* (4, 1942)

Foot, Nathan Chandler, M.D. 340 E. 72nd St., New York City. *Professor of Surgical Pathology, Cornell University Medical College; Surgical Pathologist, New York Hospital.* (4, 1924)

Forbes, Alexander, A. M., M.D. Harvard Medical School, Boston, Mass. *Professor of Physiology; Member of the National Academy of Sciences.* (1, 1910)

Forbes, Ernest B., Ph.D. State College, Pa: *Director of the Institute of Animal Nutrition.* (1, 1917; 5, 1935)

Forbes, Henry S., M.D. Forest St., Milton, Mass. *Associate in Neuropathology, Harvard Medical School.* (1, 1931)

Forbes, John C., M.A., Ph.D. Medical College of Virginia, Richmond. *Associate Professor of Biochemistry.* (2, 1937)

Foster, G. I , Ph.D. College of Physicians and Surgeons, 630 W. 168th St., New York City. *Associate Professor of Biological Chemistry.* (2, 1923)

Foster, Harry E., M.D. Cutter Laboratory, Berkeley, Calif. *Medical Director.* (6, 1913)

Foster, Robert H. K., Ph.D., M.D. 160 Church St., Nutley, N. J. *Pharmacologist, Hoffman-La Roche, Inc.* (1, 1940)

Fraenkel-Conrat, Heinz, M.D., Ph.D. Institute of Experimental Biology, University of California, Berkeley. *Research Associate.* (2, 1942)

Francis, Thomas, Jr., M.D., M.S., Sc.D. (hon.). School of Public Health, University of Michigan, Ann Arbor. *Professor of Epidemiology.* (4, 1940; 6, 1930)

Franke, Florent E., M.D. 9 Sylvester, Webster Groves, Mo. *Assistant Professor of Physiology, St. Louis University School of Medicine.* (1, 1934)

Frankel, Edward M., Ph.D. Old Tyme Distillers, Inc., Cedarhurst, Md. (2, 1916).

Fraser, Alexander MacLeod, A.M., M.D., C.M. McGill University, Montreal, Canada. *Lecturer in Pharmacology.* (3, 1939)

Fraser, Donald T., M.B. Connaught Laboratories, University of Toronto, Toronto 5, Canada. *Professor of Hygiene and Preventive Medicine.* (6, 1935)

Freeman, Harry, M.D. Worcester State Hospital, Worcester, Mass. *Internist, Research Service.* (1, 1939)

Freeman, Norman, E., M.D. University of Pennsylvania Medical School, Philadelphia. *J. Wm. White Assistant Professor of Research Surgery.* (1, 1936)

Freeman, Smith, M.D., Ph.D. Northwestern University School of Medicine, 303 E. Chicago Ave., Chicago, Ill. *Assistant Professor of Physiology and Pharmacology.* (1, 1937)

Freund, Jules, M.D. Research and Antitoxin Laboratory, Otisville, N. Y. *Assistant Director.* (4, 1930; 6, 1924)

Friedgood, Harry B., M.D. Harvard Medical School, Boston, Mass. *Instructor in Medicine.* (1, 1936)

Friedemann, Theodore E., M.A., Ph.D. Northwestern University Medical School, 303 E.

Chicago Ave., Chicago, Ill. *Associate Professor of Physiology.* (2, 1925)

Friedewald, William F., M.D. International Health Division, The Rockefeller Foundation, 66th St. and York Ave., New York City. *Member of Staff.* (4, 1941)

Friedman, Maurice H., Ph.D., M.D. 5054 Woodlawn Ave., Chicago, Ill. (1, 1929)

Friedman, M. H. F., M.A., Ph.D. Jefferson Medical College of Philadelphia, 1025 Walnut St., Philadelphia, Pa. *Associate in Physiology.* (1, 1941)

Friedman, Nathan B., M.D. Stanford University School of Medicine, San Francisco, Calif. *Instructor in Pathology.* (4, 1942)

Frisch, Arthur W., Ph.D., M.D. College of Medicine, Wayne University, Detroit, Mich. *Instructor.* (6, 1938)

Fruton, J. S., Ph.D. Rockefeller Institute, 66th St. and York Ave., New York City. *Associate in Chemistry.* (2, 1938)

Fulton, John Farquhar, M.A., Ph.D., M.D. Yale University School of Medicine, New Haven, Conn. *Sterling Professor of Physiology.* (1, 1925)

Funk, Casimir, D.Sc., Ph.D. 250 E. 43rd St., New York City. (2, 1921)

Furth, Jacob, M.D. Cornell University Medical College, 1300 York Ave., New York City. *Associate Professor of Pathology.* (4, 1932; 6, 1930)

Gaebler, Oliver H., Ph.D., M.D. Henry Ford Hospital, Detroit, Mich. *Associate in Chemistry.* (2, 1927)

Gaffron, Hans, Ph.D. Department of Chemistry, University of Chicago, Chicago, Ill. *Research Associate in Chemistry and Biochemistry.* (2, 1941)

Gager, C. Stuart, Ph.D., Sc.D., Pd.D. 1000 Washington Ave., Brooklyn, N. Y. *Director, Brooklyn Botanic Garden.* (2, 1906)

Gagge, Adolf Pharo, Ph.D. Aeromedical Research Laboratory, Wright Field, Dayton, O. *Major, Air Corps, U. S. Army; on leave from Yale University and John B. Pierce Laboratory of Hygiene.* (1, 1939)

Galambos, Robert, M.A., Ph.D. Harvard Medical School, Boston, Mass. *Research Fellow in Physiology (Harvard Medical School); Tutor in Biochemistry (Harvard College).* (1, 1942)

Gall, Edward A., M.D. Bethesda Hospital, Cincinnati, O. *Assistant Professor of Pathology, College of Medicine, University of Cincinnati.* (4, 1941)

Gallagher, Thomas F., Ph.D. University of Chicago, Chicago, Ill. *Associate Professor of Biochemistry.* (2, 1932)

Gallup, Willis D., M.S., Ph.D. Oklahoma Agricultural and Mechanical College, Stillwater. *Chemist and Professor of Agricultural Chemistry.* (2, 1932)

Gamble, James L., M.D., S.M. 33 Edgehill Rd., Brookline, Mass. *Professor of Pediatrics, Harvard Medical School.* (2, 1922; 5, 1933)

Gantt, W. Horsley, M.D. Phipps Psychiatric Clinic, Johns Hopkins Hospital, Baltimore, Md. *Associate in Psychiatry.* (1, 1935)

Garbat, Abraham L., M.D. 103 E. 78th St., New York City. *Attending Physician, Lenox Hill Hospital.* (6, 1913)

Gardner, Leroy U., M.D. Saranac Laboratory for Study of Tuberculosis, Saranac Lake, N. Y. *Director of the Trudeau Foundation.* (4, 1927)

Garrey, Walter Eugene, Ph.D., M.D. Vanderbilt University School of Medicine, Nashville, Tenn. *Professor of Physiology.* (1, 1910; 2, 1906)

Gasser, Herbert S., A.M., M.D., Sc.D. (hon.) Rockefeller Institute for Medical Research, 66th St. and York Ave., New York City. *Director of Laboratories; Member of the National Academy of Sciences.* (1, 1915; 3, 1924)

Gates, Olive, M.D. Huntington Memorial Hospital, 695 Huntington Ave., Boston, Mass. *Associate Pathologist.* (4, 1940)

Gaunt, Robert, Ph.D. Washington Square College, New York University, New York City. *Assistant Professor of Biology.* (1, 1939)

Gay, Leslie N., M.D. 1114 St. Paul St., Baltimore, Md. *Director of the Allergy Clinic, Johns Hopkins Hospital; Visiting Physician to Johns Hopkins Hospital; Associate in Medicine, Johns Hopkins University.* (6, 1927)

Geiling, E. M. K., M.S., M.D., Ph.D. University of Chicago, Chicago, Ill. *Frank P. Hixor Distinguished Service Professor of Pharmacology and Chairman of Department.* (1, 1933; 2, 1927; 3, 1925)

Gelfan, Samuel, Ph.D. 54 W. Illinois St., Chicago, Ill. *Director of Research, Van Patten Pharmaceutical Co.* (1, 1930)

Gellhorn, Ernst, M.D., Ph.D. University of Illinois College of Medicine, 1853 W. Polk St., Chicago. *Professor of Physiology.* (1, 1930)

Gemmill, Chalmers L., M.D. School of Aviation Medicine, Naval Air Base, Pensacola, Fla. *Associate Professor of Physiology, Johns Hopkins Medical School (on leave).* (1, 1928; 2, 1935)

Gerard, R. W., Ph.D., M.D. University of Chicago, Chicago, Ill. *Professor of Physiology.* (1, 1927)

Gerstenberger, Henry John, M.D. Western Reserve University, Cleveland, O. *Professor of Pediatrics, School of Medicine, Western Reserve University; Director of Pediatrics, Babies and Children's Hospital.* (5, 1938)

Gesell, Robert, M.D. University of Michigan, Ann Arbor. *Professor of Physiology.* (1, 1913)

Gettler, Alexander O., A.M., Ph.D., LL.D. 400 E. 29th St., New York City. *Professor of Chemistry and Toxicology, Washington Square College of New York University.* (**2,** 1916)

Gey, George Otto, M.D. Cancer Research and Tissue Culture Laboratory, Johns Hopkins Medical School, Baltimore, Md. *Instructor in Surgery.* (**1,** 1940)

Gibbs, Frederick Andrews, M.D. Neurological Unit, Boston City Hospital, Boston, Mass. *Instructor in Neurology, Harvard Medical School.* (**1,** 1935)

Gibbs, Owen Stanley, M.B., Ch.B. (Edin.) R.F.D. 4, Box 358-A, Memphis, Tenn. *Chief, Division of Pharmacology; Professor of Pharmacology, University of Tennessee Medical School.* (**1,** 1935; **3,** 1930)

Gibson, Robert Banks, Ph.D. University Hospital, Iowa City, Iowa. *Associate Professor of Biochemistry, State University of Iowa.* (**1,** 1907; **2,** 1906)

Gilbert, Ruth, A.M., M.D. R.F.D. 2, Altamont, N. Y. *Bacteriologist, New York State Department of Health, Albany.* (**6,** 1920)

Gies, William John, M.S., Ph.D., Sc.D., LL.D., F.A.C.D. 632 W. 168th St., New York City. *Professor of Biological Chemistry, Columbia University.* (**1,** 1898; **2,** 1906; **3,** 1909)

Gilman, Alfred, Ph.D. Sterling Hall of Medicine, Yale University, New Haven, Conn. *Assistant Professor of Pharmacology and Toxicology.* (**1,** 1935; **3,** 1934)

Gilson, Arthur S., Jr., A.M., Ph.D. Washington University Medical School, St. Louis, Mo. *Associate Professor of Physiology.* (**1,** 1927)

Githens, Thomas Stotesbury, M.D. The Cambridge, Wissahickon and Chelten Aves., Germantown, Philadelphia, Pa. (**1,** 1915)

Givens, Maurice H., Ph.D. 1750 N. Ashland Ave., Chicago, Ill. *Biochemist, Northwestern Yeast Company.* (**1,** 1917; **2,** 1915)

Glaser, O. C., Ph.D. Amherst College, Amherst, Mass. *Professor of Biology.* (**1,** 1913)

Glazko, Anthony J., Ph.D. 2319 College Ave., Apt. H, Berkeley, Calif. *Research Associate in Pharmacology, University of Michigan, Ann Arbor; Naval Reserve, U. S. Naval Hospital, San Diego, Calif.* (**1,** 1942)

Glick, David, Ph.D. Mount Sinai Hospital, 5th Ave. and 100th St., New York City. *Research Chemist.* (**2,** 1936)

Goebel, Walther F., Ph.D. The Rockefeller Institute for Medical Research, 66th St. and York Ave., New York City. *Associate Member.* (**2,** 1929; **6,** 1937)

Goerner, Alfred, Ph.G., Pharm. D., M.D. Long Island College of Medicine, 350 Henry St., Brooklyn, N. Y. *Associate Professor of Biological Chemistry.* (**2,** 1939)

Goettsch, Marianne, Ph.D. School of Tropical Medicine of Columbia University, San Juan, Puerto Rico. *Assistant Professor of Chemistry.* (**2,** 1933; **5,** 1941)

Gold, Harry, M.D. 1300 York Ave., New York City. *Assistant Professor of Pharmacology, Cornell Medical College.* (**3,** 1927)

Goldblatt, Harry, M.D. Western Reserve University, Cleveland, O. *Professor of Experimental Pathology, and Associate Director, Institute of Pathology.* (**4,** 1927)

Goldfarb, Walter, M.D. Bellevue Hospital, First Ave. and 30th St., New York City. *Resident Physician.* (**1,** 1938)

Goldforb, A. J., Ph.D. College of the City of New York, New York City. *Professor of Biology.* (**1,** 1930)

Goldring, William, M.D. New York University College of Medicine, 477 First Ave., New York City. *Assistant Professor of Medicine.* (**1,** 1939)

Goldschmit, Samuel, Ph.D. University of Pennsylvania Medical School, Philadelphia. *Associate Professor of Physiology.* (**1,** 1919; **2,** 1915)

Goodman, Louis Sanford, M.A., M.D. Yale University School of Medicine, New Haven, Conn. *Assistant Professor of Pharmacology and Toxicology.* (**3,** 1937)

Goodner, Kenneth, Ph.D. Rockefeller Institute, 66th St. and York Ave., New York City. *Associate.* (**6,** 1932)

Goodpasture, Ernest William, M.D. Vanderbilt University Medical School, Nashville, Tenn. *Professor of Pathology,* (**4,** 1923)

Gordon, Albert S., M.S., Ph.D. Washington Square College of Arts and Sciences, New York University, New York City. *Instructor in Biology.* (**1,** 1942)

Gordon, Harry H., M.D. 525 E. 68th St., New York City. *Associate in Pediatrics, Cornell University Medical School; Associate Attending Pediatrician, New York Hospital; Medical Officer, U. S. Dept. Labor.* (**5,** 1940)

Gordon, William G., M.A., Ph.D. Eastern Regional Research Laboratory, U. S. Department of Agriculture, Chestnut Hill Station, Philadelphia, Pa. *Associate Chemist.* (**2,** 1939)

Goss, Harold, Ph.D. University of California College of Agriculture, Davis. *Associate Professor in Animal Husbandry.* (**2,** 1936; **5,** 1933)

Gottschall, Russell Y., M.S., Ph.D. Bureau of Laboratories, Michigan Department of Health, Lansing. *Bacteriologist.* (**6,** 1939)

Goudsmit, Arnoldus, Jr., M.D., Ph.D. Doctors Hospital, 17th and Summer Sts., Philadelphia, Pa. *Director of Laboratories; Research Assistant, Philadelphia General Hospital.* (**1,** 1940)

Grabfield, G. Philip, M.D. The Longwood Medical Building, 319 Longwood Ave., Boston, Mass.

Associate in Medicine and Pharmacology, Harvard Medical School. (At present on leave of absence; Col. M. C., U. S. Army.) (3, 1923)

Grady, Hugh G., M.D. Fitzgerald-Mercy Hospital, Darby, Pa. *Director of Laboratories.* (4, 1940)

Graef, Irving, M.D. New York University College of Medicine, New York City. *Associate Professor of Pathology; Pathologist, Bellevue Hospital and New York University Clinic.* (4, 1941)

Graham, Clarence H., Ph.D. Brown University, Providence, R. I. *Professor of Psychology.* (1, 1933)

Graham, Helen Tredway, A.M., Ph.D. Euclid Ave. and Kingshighway, St. Louis, Mo. *Associate Professor of Pharmacology, Washington University School of Medicine.* (1, 1933; 3, 1931)

Grant, R. Lorimer, M.S., Ph.D. Division of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D. C. *Pharmacologist.* (2, 1938)

Graubard, Mark, M.A., Ph.D. Department of Biology, Clark University, Worcester, Mass. *Research Associate.* (1, 1940)

Grauer, Robert C., M.D. Allegheny General Hospital, Pittsburgh, Pa. *Head of Department of Research in Endocrinology and Metabolism, William H. Singer Memorial Research Laboratory; Lecturer in Pathology, School of Medicine, University of Pittsburgh.* (4, 1941)

Graves, Stuart, M.D. School of Medicine, University of Alabama, University. *Dean and Professor of Pathology.* (4, 1925; 6, 1918)

Gray, John S., M.S., Ph.D. Research Division, School of Aviation Medicine, Randolph Field, Texas. *Assistant Professor of Physiology, Northwestern University Medical School, Chicago, Ill. (on leave).* (1, 1937)

Gray, Samuel H., M.D. The Jewish Hospital of St. Louis, Kingshighway and Forest Park Blvd., St. Louis, Mo. *Pathologist, Jewish Hospital; Director of Laboratories, City Hospital.* (4, 1939)

Greaves, J. D., M.S., Ph.D. Washington University School of Medicine, Scott and Euclid Aves., St. Louis, Mo. *Instructor in Biological Chemistry.* (2, 1938)

Greaves, Joseph E., Ph.D. Utah State Agricultural College, Logan. *Professor and Head of Department of Bacteriology and Biochemistry.* (2, 1940)

Greeley, Paul O., A.M., Ph.D., M.D. University of Southern California Medical School, University Park, Los Angeles. *Associate Professor of Medical Physiology.* (1, 1940)

Green, Arda A., M.D. School of Medicine, Washington University, St. Louis, Mo. *Research Associate in Pediatrics.* (2, 1932)

Green, Daniel, M.S., M.D. Camp Sutton Station Hospital, Charlotte Substation, Charlotte, N. C. *Instructor, Pharmacology and Therapeutics, University of Tennessee (on leave); Chief of the Medical Service, Camp Sutton Station Hospital, Charlotte Substation, U. S. Army.* (3, 1942)

Green, David E., Ph.D. Department of Medicine, College of Physicians and Surgeons, Columbia University, New York City. *Associate in Biochemistry.* (2, 1941)

Green, Harold David, M.D. Western Reserve University, School of Medicine, Cleveland, O. *Associate Professor of Physiology.* (1, 1936)

Green, Robert, M.A., M.D. 223 Millard Hall, University of Minnesota, Minneapolis. *Professor of Bacteriology and Immunology.* (6, 1930)

Greenberg, David Morris, Ph.D. University of California, Berkeley. *Professor of Biochemistry.* (2, 1927)

Greene, Carl Hartley, Ph.D., M.D. 140 E. 54th St., New York City. *Associate Professor of Clinical Medicine, New York Post-Graduate Medical School of Columbia University; Clinical Professor of Medicine, Long Island College of Medicine.* (1, 1921; 2, 1922; 4, 1924)

Greene, Charles Wilson, A.M., Ph.D. 814 Virginia Ave., Columbia, Mo. *Lecturer in Physiology, Stanford University; Professor Emeritus of Physiology and Pharmacology, University of Missouri.* (1, 1900; 2, 1919; 3, 1909)

Greene, Harry S. N., M.D. Department of Pathology, Yale University School of Medicine, New Haven, Conn. *Associate Professor of Surgery and Pathology.* (4, 1937)

Greene, James Alexander, M.D. Medical School, State University of Iowa, University Hospital, Iowa City. *Associate Professor of the Theory and Practice of Medicine.* (1, 1939)

Greene, Ronald R., M.S., M.D. Northwestern University Medical School, 303 E. Chicago Ave., Chicago, Ill. *Instructor in Physiology; Instructor in Obstetrics and Gynecology.* (1, 1941)

Greengard, Harry, Ph.D., M.D. Northwestern University Medical School, 303 E. Chicago Ave., Chicago, Ill. *Assistant Professor of Physiology.* (1, 1939)

Greenstein, Jesse P., M.S., Ph.D. National Cancer Institute, Bethesda, Md. *Senior Biochemist.* (2, 1935)

Greenwald, Isidor, Ph.D. 477 First Ave., New York City. *Associate Professor of Chemistry, New York University College of Medicine.* (2, 1912; 5, 1933)

Greep, Roy O., Ph.D. Squibb Institute for Medical Research, New Brunswick, N. J. *Research Associate in Pharmacology.* (1, 1940)

Greer, C. M., M.S. Vanderbilt University School of Medicine, Nashville, Tenn. *Research Associate in Pharmacology.* (3, 1938)

Gregersen, Magnus I., A.M., Ph.D. College of Physicians and Surgeons, Columbia University, 630 W. 168th St., New York City. *Professor of Physiology.* (1, 1933)

Gregg, Donald Eaton, M.S., Ph.D. Department of Medicine, Western Reserve Medical School, 2109 Adelbert Rd., Cleveland, O. *Associate Professor of Physiology.* (1, 1933)

Greisheimer, Esther M., Ph.D., M.D. Woman's Medical College, East Falls, Philadelphia, Pa. *Professor of Physiology.* (1, 1925)

Griffin, Angus, Ph.D. Department of Bacteriology, George Washington University School of Medicine, 1335 H St., N.W., Washington, D. C. *Assistant Professor of Bacteriology.* (6, 1940)

Griffith, Fred R., Jr., M.A., Ph.D. 24 High St., Buffalo, N. Y. *Professor of Physiology, University of Buffalo Medical School.* (1, 1923; 5, 1933)

Griffith, Wendell H., M.S., Ph.D. 1402 S. Grand Blvd., St. Louis, Mo. *Major, Sanitary Corps, U. S. Army. On leave as Professor of Biological Chemistry, St. Louis University School of Medicine.* (2, 1923; 5, 1934)

Groat, William A., M.D. 713 E. Genesee St., Syracuse, N. Y. *Professor of Clinical Pathology, Syracuse University College of Medicine.* (6, 1917)

Grollman, Arthur, M.D., Ph.D. Dept. of Medicine, Bowman Gray School of Medicine of Wake Forest College, Winston-Salem, N. C. *Research Professor of Medicine; Associate Professor of Biochemistry.* (1, 1928; 3, 1933)

Gross, Erwin G., Ph.D., M.D. Medical Laboratories, State University of Iowa, Iowa City. *Professor of Pharmacology.* (1, 1927; 2, 1923; 3, 1927)

Gross, Robert E., M.D. Harvard Medical School, 300 Longwood Ave., Boston, Mass. *Assistant Professor of Surgery.* (4, 1940)

Gruber, Charles M., A.M., M.D., Ph.D. Jefferson Medical College, 1025 Walnut St., Philadelphia, Pa. *Professor of Pharmacology.* (1, 1914; 3, 1919)

Gruhzit, Oswald M., M.D. Research Laboratories, Parke, Davis & Co., Detroit, Mich. *Research in Pathology and Pharmacology.* (4, 1928)

Grundfest, Harry, A.M., Ph.D. Rockefeller Institute for Medical Research, 66th St. and York Ave., New York City. *Assistant in Physiology.* (1, 1932)

Gudernatsch, F., Ph.D. Graduate School, New York University, Washington Square E., New York City. *Visiting Professor.* (1, 1930)

Guerrant, N. B., M.S., Ph.D. Pennsylvania State College, State College. *Professor of Biological Chemistry.* (2, 1934; 5, 1933)

Guest, George Martin, M.S., M.D. The Children's Hospital, Research Foundation, Elland and Bethesda Aves., Cincinnati, O. *Fellow of the Children's Hospital Research Foundation; Associate Professor of Pediatrics, University of Cincinnati, College of Medicine and Graduate School.* (2, 1933)

Gulick, Addison, A.M., Ph.D. 308 Westmount Ave., Columbia, Mo. *Professor of Physiological Chemistry, University of Missouri.* (1, 1915; 5, 1933)

Gunn, Francis D., M.D. Northwestern University School of Medicine, 303 E. Chicago Ave., Chicago, Ill. *Associate Professor of Pathology.* (4, 1938)

Gurin, S., M.S., Ph.D. University of Pennsylvania School of Medicine, Philadelphia. *Assistant Professor of Physiological Chemistry.* (2, 1938)

Gustavson, Reuben G., Ph.D. University of Colorado, Boulder. *Professor of Chemistry.* (2, 1927)

Gustus, Edwin L., Ph.D. The Wilson Laboratories, 4221 S. Western Ave. Blvd., Chicago, Ill. *Assistant to General Manager.* (2, 1934)

Guthrie, Charles Claude, M.D., Ph.D., Sc.D. University of Pittsburgh Medical School, Pittsburgh, Pa. *Professor of Physiology and Pharmacology.* (1, 1905; 3, 1909)

de Gutiérrez-Mahoney, C. G., M.D. Vanderbilt University School of Medicine, Nashville, Tenn. *Associate Professor of Neurology.* (1, 1940; 4, 1941)

György, Paul, M.D. Babies' and Children's Hospital, Western Reserve University, 2103 Adelbert Rd., Cleveland, O. *Associate Professor of Pediatrics.* (2, 1938; 5, 1939)

Haag, Harvey B., M.D. Medical College of Virginia, Richmond. *Professor of Pharmacology.* (3, 1934)

Haag, J. R., Ph.D. Oregon Agricultural Experiment Station, Corvallis. *Chemist.* (5, 1941)

Hadley, Philip Bardwell, Ph.D. Institute of Pathology, Western Pennsylvania Hospital, Pittsburgh. *Chief of Bacteriological Service and Research Bacteriologist.* (4, 1927)

Hafkesbring, H. Roberta, Ph.D. Woman's Medical College of Pennsylvania, East Falls, Philadelphia. *Associate Professor of Physiology.* (1, 1931)

Haggard, Howard W., M.D. 4 Hillhouse Ave., New Haven, Conn. *Director of the Laboratory of Applied Physiology, Yale University.* (1, 1919; 2, 1920)

Hahn, Paul F., Ph.D. University of Rochester School of Medicine, Rochester, N. Y. *Associate in Biochemistry and Experimental Pathology.* (4, 1939)

Haig, Charles, M.A., Ph.D. Research Service, Columbia Division, Welfare Hospital, Welfare Island, New York City. *Research Fellow.* (1, 1942)

Haldi, John, A.M., Ph.D. Emory University, Atlanta, Ga. *Associate Professor of Physiology.* (1, 1928)

Hale, Worth, M.D. Harvard Medical School, Boston, Mass. *Associate Professor of Pharmacology.* (1, 1908; 3, 1908)

Hale, Wm. M., M.D. The State University of Iowa College of Medicine, Iowa City. *Professor of Bacteriology.* (4, 1941; 6, 1935)

Hall, F. G., M.A., Ph.D. Duke University, Durham, N. C. *Professor of Zoology.* (1, 1937)

Hall, George Edward, M.D., Ph.D. Banting Institute, University of Toronto, Toronto, Canada. *Associate Professor.* (1, 1938)

Hall, Victor E., M.A., M.D. Department of Physiology, Stanford University, Calif. *Associate Professor of Biology.* (1, 1934)

Halliday, Nellie, Ph.D. Research Laboratory, Mt. Zion Hospital, San Francisco, Calif. (5, 1933)

Halpert, Béla, M.D. University of Oklahoma School of Medicine, Oklahoma City. *Director of Laboratories and Professor of Clinical Pathology.* (4, 1936)

Halsey, John T., M.D. P. O. Box 264, Waveland, Miss. *Professor Emeritus of Pharmacology, Tulane University of Louisiana.* (3, 1929)

Halstead, Ward C., M.A., Ph.D. Dept. of Medicine, University of Chicago, Chicago, Ill. *Assistant Professor Experimental Psychology, Division of Psychiatry.* (1, 1942)

Ham, Arthur W., M.D. University of Toronto, Toronto 5, Canada. *Associate Professor of Anatomy; in charge of Histology.* (4, 1939)

Hambourger, Walter E., Ph.D., M.D. G. D. Searle & Co. (Smokie, Ill.), P. O. Box 5110, Chicago, Ill. *Pharmacologist.* (3, 1934)

Hamilton, Bengt L. K., M.D., Sc.D. 826 E. 61st St., Chicago, Ill. (2, 1925)

Hamilton, James B., Ph.D. Yale University School of Medicine, 333 Cedar St., New Haven, Conn. *Assistant Professor of Anatomy.* (1, 1938)

Hamilton, Tom S., M.S., Ph.D. 551 Old Agricultural Building, Urbana, Ill. *Professor of Animal Nutrition, University of Illinois.* (2, 1937; 5, 1938)

Hamilton, W. F., Ph.D. University of Georgia School of Medicine, Augusta. *Professor of Physiology and Pharmacology.* (1, 1924)

Hammett, Frederick S., M.S., A.M., Ph.D. 493 Commercial St., Provincetown, Mass. *Scientific Director, Lankenau Hospital Research Institute, Philadelphia, Pa.* (1, 1920; 2, 1917)

Hampel, C. W., Ph.D. School of Medicine, American University of Beirut, Beirut, Lebanese Republic. *Associate Professor of Physiology.* (1, 1936)

Handley, Carroll A., Ph.D. Western Regional Research Laboratory, Albany, Calif. *Associate Pharmacologist.* (3, 1942)

Haney, Hance F., Ph.D., M.D. University of Oregon Medical School, Portland. *Professor of Physiology and Head of the Department.* (1, 1939)

Hanger, Franklin, M.D. College of Physicians and Surgeons, 630 W. 168th St., New York City. *Associate Professor of Medicine, Columbia University.* (6, 1930)

Hanke, Martin E., Ph.D. University of Chicago, Chicago, Ill. *Associate Professor of Biochemistry.* (2, 1925)

Hanke, Milton Theo., Ph.D. 7550 S. Green St., Chicago, Ill. *Research Consultant, Biochemistry and Nutrition.* (2, 1919)

Hansen, Arild E., M.D. University of Minnesota Medical School, Minneapolis. *Professor of Pediatrics.* (4, 1941; 5, 1942)

Hanzal, Ramon Francis, M.A., Ph.D. Institute of Pathology, Western Reserve University, Cleveland, O. *Assistant Professor of Pathological Chemistry.* (2, 1935)

Hanzlik, Paul J., A.M., M.D. School of Medicine, Stanford University, Sacramento and Webster Sts., San Francisco, Calif. *Professor of Pharmacology.* (1, 1912; 3, 1912)

Hardy, James Daniel, A.M., Ph.D. Russell Sage Institute of Pathology, 525 E. 68th St., New York City. *Research Associate.* (1, 1939)

Hardy, Mary, D.Sc. The Brearley School, 610 E. 83rd St., New York City. *Teacher of Science.* (1, 1933)

Hare, Kendrick, Ph.D. State University of Iowa, Iowa City. *Assistant Professor of Anatomy.* (1, 1938)

Harger, R. N., M.A., Ph.D. Indiana University School of Medicine, Indianapolis. *Professor of Biochemistry and Toxicology.* (2, 1938)

Harkins, Henry Nelson, M.S., Ph.D., M.D. 7433 Poe Ave., Detroit, Mich. *Associate Surgeon, Henry Ford Hospital, Detroit.* (1, 1942)

Harmon, Paul M., A.M., Ph.D. Indiana University, Bloomington. *Professor of Physiology.* (1, 1930)

Harne, O. G. University of Maryland School of Medicine, Baltimore. *Associate Professor of Histology.* (1, 1935)

Harned, Ben King, M.S., Ph.D. Woman's Medical College of Pennsylvania, East Falls, Philadelphia. *Professor of Pharmacology.* (2, 1931; 3, 1941)

Harris, Albert H., 2nd, M.D. Loudenville, N. Y. (6, 1937)

Harris, Albert Sidney, Ph.D. Western Reserve University School of Medicine, Cleveland, O. *Assistant Professor of Physiology.* (**1,** 1939)

Harris, Milton, Ph.D. National Bureau of Standards, Washington, D. C. *Director of Research, Textile Foundation Research Associateship.* (**2,** 1939)

Harris, Robert S. Massachusetts Institute of Technology, Cambridge. *Assistant Professor of Nutritional Biochemistry.* (**5,** 1941)

Harris, William H., M.D. Tulane University School of Medicine, New Orleans, La. *Assistant Professor of Pathology and Bacteriology.* (**4,** 1925)

Harrison, Frank, M.S., Ph.D. University of Tennessee College of Medicine, Memphis. *Assistant Professor in Anatomy.* (**1,** 1941)

Harrison, Ross Granville, M.D., Ph.D., Sc.D. Osborn Zoological Laboratory, New Haven, Conn. *Sterling Professor of Biology, Emeritus, Yale University; Chairman of the National Research Council; Member of the National Academy of Sciences.* (**1,** 1906)

Harrison, R. Wendell, M.S., Ph.D. Ricketts Laboratory, University of Chicago, Chicago, Ill. *Associate Professor of Bacteriology.* (**6,** 1934)

Harrop, George A., M.D. The Squibb Institute for Medical Research, New Brunswick, N. J. *Director.* (**2,** 1922)

Harrow, Benjamin, M.A., Ph.D. College of the City of New York, Convent Ave. and 139th St. *Professor of Chemistry.* (**2,** 1927)

Hart, E. B., B.S. Agricultural College, Madison, Wis. *Professor of Biochemistry, University of Wisconsin.* (**2,** 1910; **5,** 1933)

Hartley, Geo., Jr., M.A., Ph.D., M.D. Department of Pathology, Boston University School of Medicine, Boston, Mass. *Assistant Professor.* (**4,** 1941)

Hartline, H. K., M.D. Johnson Foundation, University of Philadelphia, Philadelphia, Pa. *Assistant Professor of Biophysics.* (**1,** 1929)

Hartman, Carl G., A.M., Ph.D. Department of Zoology, University of Illinois, Urbana. *Professor of Zoology and Head of the Department; Member, National Academy of Sciences.* (**1,** 1921)

Hartman, Frank Alexander, A.M., Ph.D. College of Medicine, Ohio State University, Columbus. *Professor of Physiology.* (**1,** 1916)

Hartman, F. W., M.D. Henry Ford Hospital, Detroit, Mich. *Pathologist.* (**4,** 1927)

Hartmann, Alexis F., M.S., M.D. 500 S. Kingshighway, St. Louis, Mo. *Professor of Pediatrics, Washington University School of Medicine.* (**2,** 1932)

Harvey, E. Newton, Ph.D. Guyot Hall, Princeton, N. J. *Henry Fairfield Osborn Professor of Biology, Princeton University; Member, National Academy of Sciences.* (**1,** 1914; **2,** 1916)

Hass, George, M.D. Cornell University Medical College, 1300 York Ave., New York City. *Assistant Professor of Pathology.* (**4,** 1939)

Hastings, A. Baird, Ph.D., Sc.D. Harvard Medical School, Boston, Mass. *Hamilton Kuhn Professor of Biological Chemistry; Member National Academy of Sciences.* (**1,** 1927; **2,** 1921; **5,** 1940)

Hatcher, Robert A., Ph.M., M.D., D.Sc. 1300 York Ave., New York City. *Professor of Pharmacology Emeritus, Cornell University Medical College.* (**1,** 1904; **2,** 1906; **3,** 1908)

Haterius, Hans O., Ph.D. Wayne University College of Medicine, Detroit, Mich. *Professor of Physiology.* (**1,** 1936)

Hauck, Hazel M., Ph.D. Cornell University, Ithaca, N. Y. *Professor of Home Economics.* (**5,** 1941)

Hauge, Siegfred M., Ph.D. Purdue University Agricultural Experiment Station, Lafayette, Ind. *Research Associate in Biochemistry.* (**5,** 1933)

Haury, Victor G., M.D. Jefferson Medical College, Philadelphia, Pa. *Associate Professor of Pharmacology.* (**3,** 1939)

Haven, Frances L., M.A., Ph.D. University of Rochester School of Medicine and Dentistry, 260 Crittenden Blvd., Rochester, N. Y. *Associate in Biochemistry.* (**2,** 1941)

Hawk, Philip B., M.S., Ph.D. 48–14 33rd St., Long Island City, N. Y. *President, Food Research Laboratories, Inc.* (**1,** 1903; **2,** 1906)

Hawkins, William Bruce, M.D. University of Rochester School of Medicine and Dentistry, 260 Crittenden Blvd., Rochester, N. Y. *Associate Professor of Pathology.* (**4,** 1933)

Hawley, Estelle E., Ph.D. Medical School, University of Rochester, Rochester, N. Y. *Research Fellow in Pediatrics.* (**5,** 1935)

Hayman, J. M., Jr., M.D. Lakeside Hospital, Cleveland, O. *Professor of Clinical Medicine and Therapeutics, Western Reserve University.* (**1,** 1928; **3,** 1932)

Haynes, Florence W., M.A., Ph.D. Harvard Medical School, 25 Shattuck St., Boston, Mass. *Research Fellow in Medicine.* (**1,** 1937)

Haythorn, Samuel R., M.D. Allegheny General Hospital, 320 E. North Ave., Pittsburgh, Pa. *Director of William H. Singer Memorial Laboratory.* (**4,** 1925)

Haywood, Charlotte, A.M., Ph.D. Mount Holyoke College, South Hadley, Mass. *Professor of Physiology.* (**1,** 1939)

Hazen, Elizabeth L., M.A., Ph.D. New York State Department of Health Laboratories, 339 E. 25th St., New York City. *Senior Bacteriologist.* (**6,** 1931)

Heard, R. D. H., M.A., Ph.D. Dalhousie University, Halifax, Nova Scotia. *Assistant Professor of Biochemistry.* (2, 1938)

Hecht, Selig, Ph.D. Columbia University, New York City. *Professor of Biophysics.* (1, 1920)

Heft, Hattie L., Ph.D. Teachers College, Columbia University, New York City. *Assistant Professor of Physiological Chemistry.* (2, 1927)

Hegnauer, Albert H., Ph.D. Syracuse University, Syracuse, N. Y. *Assistant Professor of Physiology.* (1, 1937)

Heidelberger, Michael, Ph.D., M.A. 620 W. 168th St., New York City. *Associate Professor of Biological Chemistry, Columbia University; Chemist to the Medical Service, Presbyterian Hospital.* (2, 1927; 6, 1935)

Heilbrunn, Lewis Victor, Ph.D. University of Pennsylvania, Philadelphia. *Associate Professor of Zoology.* (1, 1930)

Heim, J. William, Ph.D. Aero-Medical Research Laboratory, Army Air Forces, Wright Field, Dayton, O. *Assistant Director, Aero-Medical Research Laboratory; Assistant in Physiology, Harvard School of Public Health.* (1, 1936)

Heinbecker, Peter, M.D. Washington University Medical School, St. Louis, Mo. *Associate Professor of Surgery.* (1, 1930)

Helff, O. M., M.S., Ph.D. New York University, University Heights, New York City. *Associate Professor of Biology.* (1, 1932)

Hellbaum, Arthur A., M.A., Ph.D. University of Oklahoma School of Medicine, Oklahoma City. *Assistant Professor of Physiology.* (1, 1937)

Hellebrandt, Frances Anna, M.D. Wisconsin General Hospital, Madison. *Research Associate in Physiology and Associate Professor of Physical Therapy, University of Wisconsin.* (1, 1933)

Heller, Victor G., Ph.D. Oklahoma A. & M. College, Stillwater. *Professor and Head of the Department of Agricultural Chemistry Research.* (2, 1935; 5, 1935)

Hellerman, Leslie, Ph.D. Johns Hopkins University School of Medicine, 710 N. Washington St., Baltimore, Md. *Associate in Physiological Chemistry.* (2, 1935)

Helmer, Oscar Marvin, M.S., Ph.D. Lilly Laboratory for Clinical Research, The Indianapolis City Hospital, Indianapolis, Ind. *Head of Department of Physiological Chemistry; Research Associate in the Department of Medicine, Indiana University School of Medicine.* (2, 1935)

Hemingway, Allan, Ph.D. 210 Millard Hall, University of Minnesota, Minneapolis. *Assistant Professor of Physiological Chemistry; Temporarily at School of Aviation Medicine, Randolph Field, Texas.* (1, 1933)

Henderson, Velyien E., M.A., M.B., F.R.S.C. Medical Bldg., University of Toronto, Toronto, Ont., Canada. *Professor of Pharmacology and Pharmacy.* (1, 1905; 3, 1911)

Henderson, Yandell, Ph.D., M.D. (hon.). Yale University, New Haven, Conn. *Professor of Physiology Emeritus; Member, National Academy of Sciences.* (1, 1900; 2, 1919; 3, 1911)

Hendrix, Byron M., Ph.D. School of Medicine, University of Texas, Galveston. *Professor of Biochemistry.* (2, 1920)

Hendrix, James Paisley, M.A., M.D. Duke Hospital, Durham, N. C. *Associate in Medicine (in charge of Therapeutics); Associate in Physiology and Pharmacology, Duke University School of Medicine.* (3, 1942)

Hendry, Jessie L., M.A. Division of Laboratories and Research, New York State Department of Health, New Scotland Ave., Albany. *Senior Bacteriologist.* (6, 1938)

Henle, Werner, M.D. University of Pennsylvania, Philadelphia. *Assistant Professor of Bacteriology in Pediatrics.* (6, 1938)

Hepburn, Joseph Samuel, A.M., M.S., Ph.D., M.D. 235 N. 15th St., Philadelphia, Pa. *Professor of Chemistry and Research Associate in Gastro-Enterology, Hahnemann Medical College and Hospital.* (2, 1915)

Hepler, Opal, Ph.D., M.D. Northwestern University Medical School, 303 E. Chicago Ave., Chicago, Ill. *Assistant Professor of Pathology.* (4, 1939)

Herbst, R. M., Ph.D. Nichols Laboratory, New York University, University Heights, New York City. *Assistant Professor of Organic Chemistry.* (2, 1938)

Herrick, C. Judson, Ph.D. 236 Morningside Drive, Grand Rapids, Mich. *Professor Emeritus of Neurology, University of Chicago; Member of the National Academy of Sciences.* (1, 1907)

Herrick, Julia F., M.A., Ph.D. Fort Monmouth, N. J. *Associate Physicist, Signal Service at Large; Assistant Professor of Biophysics, University of Minnesota; Associate in the Division of Experimental Medicine, Mayo Foundation (on leave).* (1, 1933)

Herrin, Raymond C., Ph.D., M.D. University of Wisconsin Medical School, Madison. *Associate Professor of Physiology.* (1, 1932)

Herrington, Lovic P., M.A., Ph.D. 290 Congress Ave., New Haven, Conn. *Associate Director, John B. Pierce Laboratory of Hygiene; Research Associate Professor, Dept. of Public Health, Yale Medical School.* (1, 1942)

Herriott, Roger M., Ph.D. Rockefeller Institute for Medical Research, Princeton, N. J. *Associate.* (2, 1940)

Herrmann, George, Ph.D., M.D. University of Texas School of Medicine, Galveston. *Professor of Medicine.* (4, 1925)

Herrmann, Julian B., M.D. Yale School of Medicine, New Haven, Conn. *Research Assistant in Pharmacology.* (3, 1941)

Herrmann, Louis George, M.D. Cincinnati General Hospital, Cincinnati, O. *Associate Professor of Surgery, University of Cincinnati College of Medicine.* (4, 1933)

Hershey, A. D., Ph.D. Washington University School of Medicine, St. Louis, Mo. *Assistant Professor of Bacteriology and Immunology.* (2, 1942)

Hertig, Arthur T., M.D. Harvard University Medical School, 25 Shattuck St., Boston, Mass. *Assistant Professor of Pathology; Associate in Obstetrics; Pathologist to Boston Lying-in-Hospital and to Free Hospital for Women, Brookline, Mass.* (4, 1941)

Hertz, Saul, M.D. Massachusetts General Hospital, Fruit St., Boston. *Research Associate, Harvard Medical School and Massachusetts Institute of Technology.* (4, 1935)

Hertzman, Alrick B., Ph.D. St. Louis University School of Medicine, St. Louis, Mo. *Associate Professor of Physiology and Chairman of the Department.* (1, 1925)

Herwick, Robert P., Ph.D., M.D. U. S. Food and Drug Administration, Washington, D. C. *Senior Pharmacologist and Assistant Chief, Drug Division.* (3, 1938)

Hess, Charles L., M.S., M.D. 308 Davidson Bldg., Bay City, Mich. (1, 1916)

Hess, Walter C., Ph.D. Chemo-Medical Research Institute, Georgetown University, Washington, D. C. *Associate Research Professor.* (2, 1935)

Hewitt, Earl Albon, M.S., Ph.D. Iowa State College, Ames. *Associate Professor of Veterinary Physiology.* (1, 1932)

Hewitt, Julia A. W., B.A. Nassau Hospital, Mineola, N. Y. *Senior Technician, in charge.* (6, 1921)

Heyroth, Francis F., M.D., Ph.D. Kettering Laboratory, College of Medicine, University of Cincinnati, Cincinnati, O. *Assistant Professor of Applied Physiology.* (2, 1935)

Hiatt, Edwin P., M.A., Ph.D. Vanderbilt University School of Medicine, Nashville, Tenn. *Instructor in Physiology.* (1, 1942)

Higgins, Harold Leonard, M.D. Massachusetts General Hospital, Boston. *Assistant Professor of Pediatrics, Harvard University.* (1, 1914; 5, 1933)

Hill, Edgar S., M.S., Ph.D. Washington University, College of Dentistry, St. Louis, Mo. *Assistant Professor of Biochemistry and Physiology.* (2, 1936)

Hill, Robert M., M.S., Ph.D. 4200 E. 9th Ave., Denver, Colo. *Associate Professor of Biochemistry, University of Colorado Medical School.* (2, 1933)

Hill, Samuel E., M.A., Ph.D. Russell Sage College, Troy, N. Y. *Professor of Biology.* (1, 1934)

Hiller, Alma, Ph.D. Rockefeller Institute for Medical Research, 66th St. and York Ave., New York City. *Associate.* (2, 1929)

Himmelsbach, C. K., M.D. U.S.P.H.S. Hospital, Lexington, Ky. *Passed Assistant Surgeon.* (3, 1938)

Himwich, Harold E., M.D. Albany Medical College, Albany, N. Y. *Professor of Physiology and Pharmacology.* (1, 1925; 5, 1933)

Hines, Harry M., M.S., Ph.D. The State University of Iowa, Iowa City. *Professor of Physiology.* (1, 1928)

Hines, Marion, Ph.D. Johns Hopkins Medical School, Baltimore, Md. *Associate Professor of Anatomy.* (1, 1932)

Hinrichs, Marie, Ph.D., M.D. Southern Illinois Normal University, Carbondale. *Associate Professor of Physiology; Head of Student Health Service.* (1, 1928)

Hinsey, Joseph C., M.S., Ph.D. Cornell University Medical College, 1300 York Ave., New York City. *Professor of Anatomy and Dean of the Medical College.* (1, 1929)

Hisaw, Frederick L., A.M., Ph.D. The Biological Laboratories, Harvard University, Cambridge Mass. *Professor of Zoology.* (1, 1932)

Hitchcock, David I., Ph.D. 333 Cedar St., New Haven, Conn. *Associate Professor of Physiology, Yale University.* (2, 1930)

Hitchcock, Fred A., M.Sc., Ph.D. Ohio State University, Columbus. *Associate Professor of Physiology.* (1, 1927; 5, 1933)

Hitchings, George H., M.S., Ph.D. 2109 Adelbert Road, Cleveland, O. *Senior Instructor, Western Reserve University.* (2, 1942)

Hjort, Axel M., M.D., Ph.D. 14 Fern Way (P. O. Box 281), Scarsdale, N. Y. *Private practice; research work at Grasslands Hospital.* (2, 1925)

Hoagland, Charles L., M.D. Rockefeller Institute, 66th St. and York Ave., New York City. *Associate Member.* (6, 1940)

Hoagland, Hudson, M.S., Ph.D. Clark University, Worcester, Mass. *Professor of Physiology and Chairman of the Department of Biology.* (1, 1932)

Höber, Rudolf. University of Pennsylvania Medical School, Philadelphia. *Visiting Professor of Physiology.* (1, 1936)

Hodes, Robert, Ph.D. Johnson Foundation, University of Pennsylvania, Philadelphia. *Johnson Foundation Fellow in Medical Physics.* (1, 1941)

Hodge, Harold C., Ph.D. University of Rochester School of Medicine and Dentistry, Rochester, N. Y. *Associate Professor of Biochemistry and Pharmacology.* (2, 1937)

Hoff, Ebbe Curtis, M.A., Ph.D. Department of Physiology, Yale University School of Medicine, 333 Cedar St., New Haven, Conn. (1, 1933)

Hoff, Hebel E., M.A., Ph.D. Yale Medical School, New Haven, Conn. *Associate Professor of Physiology.* (1, 1933)

Hoffman, Olive, M.S., Ph.D. Presbyterian Hospital, 51 N. 39th St., Philadelphia, Pa. *Research Associate.* (1, 1935)

Hoffman, William Samuel, Ph.D., M.D. 710 S. Wolcott Ave., Chicago, Ill. *Professor of Physiological Chemistry and Associate Professor of Medicine, Chicago Medical School.* (2, 1935)

Hogan, Albert G., A.M., Ph.D. 105 Schweitzer Hall, Columbia, Mo. *Professor of Animal Nutrition, University of Missouri.* (2, 1916; 5, 1933)

Hogness, Thorfin R., Ch.E., Ph.D. George Herbert Jones Chemical Laboratory, University of Chicago, Chicago, Ill. *Professor of Chemistry.* (2, 1941)

Holck, Harald G. O., Ph.D. College of Pharmacy, University of Nebraska, Lincoln. *Associate Professor of Pharmacology.* (1, 1935; 3, 1938)

Hollander, Franklin, Ph.D. Mount Sinai Hospital, Fifth Ave. and 100th St., New York City. *Associate in Physiology; Head of Gastro-Enterology Research Laboratory.* (1, 1942; 2, 1932)

Holman, Russell Lowell, M.D. University of North Carolina School of Medicine, Chapel Hill. *Associate Professor of Pathology.* (4, 1940)

Holmes, Arthur Dunham, Ph.D. Massachusetts State College, Amherst. *Research Professor of Chemistry.* (2, 1931; 5, 1933)

Holmes, Julia O., M.S., Ph.D. Massachusetts State College, Amherst. *Research Professor of Nutrition.* (2, 1942; 5, 1936)

Holt, Joseph Paynter, M.S., M.D., Ph.D. University of Louisville School of Medicine, 101 W. Chestnut St., Louisville, Ky. *Assistant Professor of Physiology.* (1, 1942)

Holt, L. Emmett, Jr., M.D. Johns Hopkins Hospital, Baltimore, Md. *Associate Professor of Pediatrics, Johns Hopkins University.* (2, 1930)

Hoobler, Icie Macy, Ph.D. 660 Frederick St., Detroit, Mich. *Director of Research, Children's Fund of Michigan; Associate in Nutrition, Medical Staff of the Children's Hospital of Michigan.* (2, 1925; 5, 1933)

Hooker, Davenport, M.A., Ph.D. University of Pittsburgh School of Medicine, Pittsburgh, Pa. *Professor of Anatomy.* (1, 1920)

Hooker, Donald R., M.S., M.D. 19 W. Chase St., Baltimore, Md. *Lecturer in Physiological Hygiene, School of Hygiene and Public Health, Johns Hopkins University; Managing Editor of American Journal of Physiology, Physiological Reviews and Federation Proceedings.* (1, 1906; 3, 1911)

Hooker, Sanford B., A.M., M.D. 80 E. Concord St., Boston, Mass. *Member, Evans Memorial.* (6, 1918)

Hoppert, C. A., Ph.D. Michigan State College, Box 626, East Lansing. *Professor of Biological Chemistry.* (5, 1935)

Horsfall, Frank L., Jr., M.D., C.M. Rockefeller Institute, 66th St. and York Ave., New York City. *Member.* (6, 1937)

Horwitt. M. K., Ph.D. Elgin State Hospital, Elgin, Ill. *Research Biochemist and Director, Biochemical Research Laboratory; Assistant Professor of Physiological Chemistry, University of Illinois School of Medicine.* (2, 1941)

Hoskins, R. G., Ph.D., M.D. Harvard Medical School, Boston, Mass. *Research Associate in Physiology, Harvard University; Director of Research, Memorial Foundation for Neuroendocrine Research.* (1, 1911)

Hotchkiss, Rollin D., Ph.D. The Rockefeller Institute for Medical Research, 66th St. and York Ave., New York City. *Associate.* (2, 1941)

Howard, Evelyn, A.M., Ph.D. Johns Hopkins School of Medicine, Baltimore, Md. *Instructor in Physiology.* (1, 1933)

Howard, Marion E., M.D. New Haven Hospital, New Haven, Conn. *Assistant Professor of Medicine, Yale School of Medicine; Associate Physician, New Haven Hospital and New Haven Dispensary.* (4, 1939; 6, 1937)

Howe, Paul E., A.M., Ph.D. 2823 29th St. N.W., Washington, D. C. *Colonel, Sanitary Corps; Chief, Nutrition Branch, Office of the Surgeon General, U. S. Army. On leave as Chief, Animal Nutrition Division, and Assistant Chief, Bureau of Animal Industry, U. S. Department of Agriculture.* (1, 1913; 2, 1909; 5, 1933)

Howe, Percy R., M.D., D.D.S. Harvard Medical School, Boston, Mass. *Director Forsyth Dental Infirmary; Professor Dental Sciences; Instructor in Pathology.* (5, 1935)

Howell, Katherine M., M.D. Michael Reese Hospital, 2900 Ellis Ave., Chicago, Ill. *Head of Departments of Bacteriology and Serology.* (6, 1940)

Howell, Stacey F., Ph.D. V. D. Research Laboratory, U. S. Marine Hospital, Stapleton, Staten Island, N. Y. *Associate Chemist, U. S. Public Health Service.* (2, 1940)

Howell, William H., Ph.D., M.D., Sc.D., LL.D. 112 St. Dunstan's Road, Baltimore, Md. *Professor Emeritus of Physiology, Johns Hopkins University; Member, National Academy of Sciences.* (1, 1887; 2, 1912)

Hubbard, Roger Sanford, A.M., Ph.D. 100 High St., Buffalo, N. Y. *Biochemist, Buffalo General*

Hospital; Professor of Applied Physiology, Buffalo University Medical School. (**1,** 1922; **2,** 1920)

Hubbell, Rebecca B., M.S., Ph.D. Connecticut Agricultural Experiment Station, New Haven. *Assistant Biochemist.* (**2,** 1937; **5,** 1935)

Hudack, Stephen Sylvester, M.D. Presbyterian Hospital, 620 W. 168th St., New York City. *Instructor in Surgery, Columbia University; Assistant Attending Surgeon, Presbyterian Hospital.* (**4,** 1933)

Huddleston, Ora Leonard, M.D., Ph.D. University of Colorado School of Medicine, 4200 E. 9th Ave., Denver. *Instructor in Physiology.* (**1,** 1936)

Hueper, Wilhelm C., M.D. Warner Institute for Therapeutic Research, 113 W. 18th St., New York City. *Assistant Director and Principal Pathologist.* (**4,** 1940)

Huffman, C. F., M.S., Ph.D. Michigan State College, East Lansing. *Research Professor and Associate Professor in Dairy Husbandry.* (**5,** 1937)

Huggins, Charles Brenton, M.D. University of Chicago, Chicago, Ill. *Professor of Surgery.* (**1,** 1932)

Hughes, Joseph, M.D. 111 N. 49th St., Philadelphia, Pa. *Assistant Professor of Experimental Neurology, Graduate School of Medicine, University of Pennsylvania; Director of Laboratory, Pennsylvania Hospital for Mental Diseases.* (**1,** 1936)

Hughes, Josiah Simpson, M.A., M.S., Ph.D. Kansas State College, Manhattan. *Professor of Chemistry.* (**2,** 1931; **5,** 1939)

Hughes, Thomas P., A.M., Ph.D. Rockefeller Foundation, 49 W. 49th St., New York City. *Member of Staff, International Health Division.* (**6,** 1934)

Hulpieu, Harold R., M.A., Ph.D. Indiana University School of Medicine, Indianapolis. *Associate Professor of Pharmacology.* (**3,** 1939)

Hunscher, Helen A., Ph.D. Western Reserve University, 2023 Adelbert Rd., Cleveland, O. *Head of Department of Home Economics.* (**5,** 1934)

Hunt, Reid, M.D., Ph.D., Sc.D. Harvard Medical School, Boston, Mass. *Professor of Pharmacology Emeritus, Harvard University; Member, National Academy of Sciences.* (**1,** 1895; **2,** 1906; **3,** 1908)

Hunter, Andrew, M.A., M.B., F.R.S.C. University of Toronto, Toronto, Canada. *Professor of Pathological Chemistry.* (**2,** 1908)

Hunter, George, M.A., D.Sc., F.R.S.C. University of Alberta, Edmonton, Canada. *Professor of Biochemistry.* (**2,** 1924)

Hunter, Jesse E., M.S., Ph.D. Allied Mills, Inc., 7500 S. Adams St., Peoria, Ill. *Director Biological Research.* (**5,** 1936)

Hussey, Raymond, M.D. Homewood Apartments, Baltimore, Md. (**4,** 1927)

Hyde, Roscoe R., A.M., Ph.D. Johns Hopkins University, Baltimore, Md. *Professor of Immunology.* (**6,** 1939)

Ingalls, Mabel S., Ph.D. Department of Bacteriology, Albany Medical School, Albany, N. Y. *Associate Professor of Bacteriology.* (**6,** 1940)

Ingle, Dwight J., M.S., Ph.D. The Upjohn Co., Research Department, Kalamazoo, Mich. *Upjohn Research Fellow.* (**1,** 1939)

Ingraham, Raymond Clifford, Ph.D. College of Medicine, University of Illinois, 1853 W. Polk St., Chicago. *Instructor in Physiology.* (**1,** 1938)

Ingram, W. R., Ph.D. College of Medicine, The State University of Iowa, Iowa City. *Professor and Head of the Department of Anatomy.* (**1,** 1936)

Inman, Ondess L., Ph.D. Antioch College, Yellow Springs, O. *Director of C. F. Kettering Foundation for the Study of Chlorophyll and Photosynthesis; Chairman of the Department of Biology.* (**2,** 1939)

Irvin, J. Logan, Ph.D. Department of Physiological Chemistry, Johns Hopkins School of Medicine, 710 N. Washington St., Baltimore, Md. *Instructor in Physiological Chemistry.* (**2,** 1942)

Irving, Laurence, A.M., Ph.D. Swarthmore College, Swarthmore, Pa. *Professor of Experimental Biology.* (**1,** 1927)

Irwin, Marian, Ph.D. The Rockefeller Institute for Medical Research, New York City. *Associate in the Division of General Physiology.* (**1,** 1927)

Irwin, M. R., Ph.D. Department of Genetics, University of Wisconsin, Madison. *Professor of Genetics.* (**6,** 1936)

Isaacs, Raphael, M.D. 104 S. Michigan Ave., Suite 408, Chicago, Ill. *Director, Department of Hematology, Michael Reese Hospital.* (**4,** 1928)

Isenberger, R. M., M.A., M.D. University of Kansas School of Medicine, Kansas City. *Professor of Pharmacology.* (**3,** 1937)

Ivy, Andrew C., Ph.D., M.D. 303 E. Chicago Ave., Chicago, Ill. *Nathan Smith Davis Professor of Physiology and Professor of Pharmacology, Northwestern University Medical School.* (**1,** 1919; **5,** 1933)

Izquierdo, J. Joaquin, M.D. National School of Medicine, Mexico City. *Professor of Physiology in the National School of Medicine and the Escuela Medico Militar of Mexico.* (**1,** 1928)

Jackson, Dennis Emerson, A.M., Ph.D., M.D. University of Cincinnati Medical School, Eden

and Bethesda Aves., Cincinnati, O. *Professor of Pharmacology.* (1, 1910; 3, 1912)

Jackson, Eugene L., Ph.C., Ph.D. Emory University, Ga. *Associate Professor of Pharmacology, Chairman Department of Pharmacology.* (3, 1942)

Jackson, Richard W., Ph.D. Eastern Regional Research Laboratory, U. S. Department of Agriculture, Wyndmoor, Pa. *Chief of Protein Division.* (2, 1930; 5, 1933)

Jacobs, Merkel Henry, Ph.D. University of Pennsylvania, Philadelphia. *Professor of General Physiology; Member of the National Academy of Sciences.* (1, 1919)

Jacobs, Walter A., A.M., Ph.D. Rockefeller Institute, 66th St. and York Ave., New York City. *Member; Member National Academy of Sciences.* (2, 1908; 3, 1913)

Jacobson, Edmund, Ph.D., M.D. Laboratory for Clinical Physiology, 310 S. Michigan Ave., Chicago, Ill. (1, 1929)

Jaffe, Henry L., M.D. Hospital for Joint Diseases, 1919 Madison Ave., New York City. *Director of Laboratories.* (4, 1925)

Jamieson, Walter A., Sc.D.(hon.). Eli Lilly & Company, Indianapolis, Ind. *Director, Biological Division.* (6, 1927)

Jasper, Herbert H., M.A., Ph.D., D. ès Sci. Montreal Neurological Institute, 3801 University St., Montreal, Que., Canada. *Lecturer in Neuroelectrography and Director of Department of Electrophysiology.* (1, 1940)

Jeans, P. C., M.D. State University of Iowa, Iowa City. *Professor of Pediatrics.* (5, 1937)

Jensen, H., Ph.D. Research Laboratories, The Upjohn Company, Kalamazoo, Mich. (2, 1929)

Jobling, James W., M.D. Columbia University, 630 W. 168th St., New York City. *Professor of Pathology.* (4—prior to 1920)

Jochim, Kenneth E., Ph.D. Michael Reese Hospital, 29th and Ellis Ave., Chicago, Ill. *Research Associate, Cardiovascular Dept.* (1, 1942)

Johlin, J. M., Ph.D., D.Sc. Vanderbilt University School of Medicine, Nashville, Tenn. *Associate Professor of Biochemistry.* (2, 1928)

Johnson, Charles C., M.D. University of Utah School of Medicine, Salt Lake City. *Professor of Pharmacology.* (3, 1929)

Johnson, Frank H., A.M., Ph.D. Princeton University, Princeton, N. J. *Assistant Professor, Dept. of Biology.* (1, 1942)

Johnson, Joseph L., Ph.D., M.D. School of Medicine, Howard University, Washington, D. C. *Professor and Head of the Department of Physiology.* (1, 1934)

Johnson, J. Raymond, Ph.D. Long Island College of Medicine, 350 Henry St., Brooklyn, N. Y. *Assistant Professor of Physiology and Pharmacology.* (1, 1938)

Johnson, Marvin J., M.S., Ph.D. University of Wisconsin, Madison. *Associate Professor of Biochemistry.* (2, 1941)

Johnson, Robert E., M.D., Ph.D. Fatigue Laboratory, Morgan Hall, Harvard University, Boston, Mass. *Research Fellow.* (2, 1939)

Johnson, Treat B., Ph.D. Amity Road, Bethany, Westville P. O., Conn. *Sterling Professor of Chemistry, Yale University; Member National Academy of Sciences.* (2, 1910)

Johnson, Victor, Ph.D., M.D. University of Chicago, Chicago, Ill. *Associate Professor of Physiology; Dean of Students in the Division of Biology and the School of Medicine.* (1, 1933)

Johnston, Charles G., M.S., M.D. Wayne University College of Medicine, Detroit, Mich. *Professor of Surgery.* (1, 1933)

Johnston, Margaret W., M.S., Ph.D. Box 276, Ann Arbor, Mich. *Research Fellow in Nutrition, University of Michigan.* (2, 1930; 5, 1938)

Jolliffe, Norman, M.D. 22 E. 36th St., New York City. *Associate Professor of Medicine, New York University College of Medicine; Associate in Medicine, Psychiatric Division, Bellevue Hospital.* (1, 1932)

Jones, D. Breese, Ph.D. Bureau of Agricultural Chemistry and Engineering, U. S. Department of Agriculture, Washington, D. C. *Chief, Protein and Nutrition Research Division.* (2, 1920; 5, 1935)

Jones, James H., M.S., Ph.D. School of Medicine, University of Pennsylvania, Philadelphia. *Assistant Professor of Physiological Chemistry.* (2, 1928; 5, 1933)

Jones, Kenneth K., M.S., Ph.D. Northwestern University Medical School, 303 E. Chicago Ave., Chicago, Ill. *Assistant Professor of Physiology and Pharmacology.* (1, 1936)

Jones, Lloyd R., M.S., Ph.D. 1402 S. Grand Blvd., St. Louis, Mo. *Associate Professor and Chairman of Department of Bacteriology, St. Louis University School of Medicine.* (6, 1933)

Joslin, Elliott P., M.A., M.D. New England Deaconess Hospital, 81 Bay State Rd., Boston, Mass. *Director, George F. Baker Clinic.* (5, 1933)

Jukes, Thomas Hughes, Ph.D. Lederle Laboratories, Pearl River, N. Y. *Associate Director, Pharmaceutical Division.* (2, 1935; 5, 1938)

Julianelle, Louis A., Ph.D., M.A. Division of Infectious Diseases, Public Health Research Institute of the City of New York, Foot of E. 15th St. *Head of Division.* (6, 1930)

Jung, Frederic Theodore, Ph.D., M.D. Northwestern University Medical School, Chicago, Ill. *Assistant Professor of Physiology and Pharmacology.* (1, 1930)

Jungeblut, Claus W., M.D. College of Physicians and Surgeons, 630 W. 168th St., New York City. *Professor of Bacteriology, Columbia University.* (4, 1929; 6, 1926)

Kabat, Elvin A., Ph.D. The Neurological Institute, 710 W. 168th St., New York City. *Research Chemist.* (2, 1940)

Kabat, Herman, Ph.D. University of Minnesota, Minneapolis. *Instructor in Physiology.* (1, 1941)

Kahn, Reuben L., Sc.D. University of Michigan Hospital, Ann Arbor. *Director of Clinical Laboratories.* (4, 1934; 6, 1919)

Kalckar, H. M., M.D. Department of Pharmacology, Washington University School of Medicine, St. Louis, Mo. *Research Associate.* (2, 1942)

Kamm, Oliver, M.S., Ph.D. Research Laboratory, Parke, Davis & Co., Detroit, Mich. *Scientific Director.* (2, 1928)

Karpovich, Peter V., M.D., M.P.E. School of Aviation Medicine, Randolph Field, Texas. *Senior Physiologist, Research Section.* (1, 1942)

Karr, Walter G., Ph.D. Graduate School of Medicine, University of Pennsylvania, Philadelphia. *Assistant Professor of Physiological Chemistry; Chemist, Medical Clinic, University Hospital; Chemist, Abington Memorial Hospital.* (2, 1925)

Karshan, Maxwell, Ph.D. Department of Biological Chemistry, Columbia University, 630 W. 168th St., New York City. *Associate Professor of Biochemistry.* (2, 1939)

Karsner, Howard T., M.D. Western Reserve University, 2085 Adelbert Rd., Cleveland, O. *Professor of Pathology; Director of the Institute of Pathology.* (4, prior to 1920; 6, 1925)

Katz, Gerhard, M.D. Tulane School of Medicine, New Orleans, La. *Assistant Professor of Pharmacology.* (3, 1937)

Katz, Louis Nelson, A.M., M.D. 2900 Ellis Ave., Chicago, Ill. *Director of Cardiovascular Research, Michael Reese Hospital; Professorial Lecturer in Physiology, University of Chicago.* (1, 1924)

Katzman, Philip A., Ph.D. St. Louis University School of Medicine, 1402 S. Grand Blvd., St. Louis, Mo. *Assistant Professor of Biochemistry.* (2, 1935)

Kay, H. D., Ph.D., D.Sc. National Institute for Research in Dairying, Shinfield, near Reading, England. *Director, Research Professor of Biochemistry, University of Reading.* (2, 1930)

Keeton, Robert W., M.S., M.D. University of Illinois College of Medicine, 1853 W. Polk St., Chicago. *Professor of Medicine.* (1, 1916; 3, 1924)

Kehoe, Robert A., M.D. Kettering Laboratory of Applied Physiology, College of Medicine, University of Cincinnati, Eden Ave., Cincinnati, O. *Research Professor of Physiology.* (1, 1940)

Keith, Norman M., M.D. Mayo Clinic, Rochester, Minn. *Consulting Physician, Division of Medicine, Mayo Clinic; Professor of Medicine, Mayo Foundation, University of Minnesota.* (1, 1920; 3, 1932; 4, 1924)

Keith, T. B., Ph.D. Pennsylvania State College, State College. *Assistant Professor of Animal Husbandry.* (5, 1941)

Keller, Allen D., Ph.D. University of Alabama School of Medicine, University. *Professor of Physiology and Pharmacology.* (1, 1931)

Kelser, Raymond A., Ph.D. Surgeon General's Office, War Department, Washington, D. C. *Brig. General, U. S. Army; Chief, Veterinary Service, Surgeon General's Office.* (4, 1932)

Kelsey, F. Ellis, Ph.D. University of Chicago, Chicago, Ill. *Research Associate (Instructor) in Pharmacology.* (3, 1941)

Kempner, Walter, M.D. Duke University School of Medicine, Durham, N. C. *Assistant Professor of Medicine.* (1, 1940)

Kendall, Edward C., M.S., Ph.D., D.Sc. 627 Eighth Ave., S.W., Rochester, Minn. *Professor of Biochemistry, Mayo Foundation, University of Minnesota.* (1, 1916; 2, 1913; 4, prior to 1920)

Kennard, Margaret A., M.D. Yale University School of Medicine, New Haven, Conn. *Assistant Professor of Physiology.* (1, 1934)

Kennedy, Cornelia, M.A., Ph.D. Snyder Hall, University Farm, St. Paul, Minn. *Associate Professor of Agricultural Biochemistry, University of Minnesota; Assistant Chemist, Minnesota Experiment Station.* (2, 1924; 5, 1934)

Kennedy, Robert P., M.D. Knollwood Drive, R. D. 1, Rochester, N. Y. (4, 1929)

Kenton, Harold B., Ph.D. New England Deaconess Hospital, Boston, Mass. *Bacteriologist and Director of the Blood Bank.* (6, 1934)

Kenyon, Allan T., M.D. University of Chicago, Division of Biological Sciences, 950 E. 59th St., Chicago, Ill. *Assistant Professor of Medicine.* 3, 1940)

Keresztesy, John C., M.A., Ph.D. Merck & Company, Inc., Rahway, N. J. *Head, Nutritional Research Laboratory.* (2, 1941)

Kerr, Stanley E., Ph.D. Near East College Association, 50 W. 50th St., New York City. *Professor of Biological Chemistry, American University of Beirut, Republic of Lebanon.* (2, 1937)

Kerr, Wm. J., M.D. University of California Hospital, Third and Parnassus Aves., San Francisco. *Professor of Medicine, University of California; Physician-in-Chief, University of California Hospital.* (3, 1930)

Kesten, Homer D., M.D. College of Physicians and Surgeons, Columbia University, New York

City. *Assistant Professor of Pathology.* (4, 1931)

Keys, Ancel, M.A., Ph.D., D. Phil. 51 Stadium South Tower, University of Minnesota, Minneapolis. *Professor of Physical Education and Physiology.* (1, 1939; 2, 1936)

Kidd, John G., M.D. Rockefeller Institute for Medical Research, 66th St. and York Ave., New York City. *Associate Member in Pathology and Bacteriology.* (4, 1938; 6, 1937)

Kik, M. C., Ph.D. College of Agriculture, University of Arkansas, Fayetteville. *Professor of Agricultural Chemistry.* (5, 1942)

Kilborn, Leslie G., M.A., M.D., Ph.D. West China Union University, Chengtu, Szechwan, China. *Professor of Physiology and Pharmacology.* (1, 1928)

Killian, John Allen, A.M., Ph.D. 50 E. 41st St., New York City. *Consulting Biochemist.* (2, 1921)

King, Barry G., M.A., Ph.D. College of Physicians and Surgeons, Columbia University, 630 W. 168th St., New York City. *Assistant Professor of Physiology.* (1, 1938)

King, Charles Edwin, Ph.D. Vanderbilt University, Nashville, Tenn. *Associate Professor of Physiology.* (1, 1916)

King, Charles Glen, Ph.D. Nutrition Foundation, Inc., Chrysler Building, New York City. *Scientific Director; Professor of Chemistry, University of Pittsburgh.* (2, 1931; 5, 1933)

King, Jessie Luella, Ph.D. Goucher College, Baltimore, Md. *Professor of Physiology.* (1, 1914)

King, Joseph T., M.D., Ph.D. 314 Millard Hall, University of Minnesota Medical School, Minneapolis. *Assistant Professor of Physiology.* (1, 1931)

King, Lester, S., M.D. The Fairfield State Hospital, Newtown, Conn. *Hospital Pathologist.* (4, 1941)

Kirk, Paul L., Ph.D. University of California, Berkeley. *Associate Professor of Biochemistry.* (2, 1933)

Kirkbride, Mary B., Sc.D. Division of Laboratories and Research, New York State Department of Health, Albany. *Associate Director.* (6, 1921)

Kleiber, M., D.Sc. University of California, Davis. *Professor of Animal Husbandry.* (5, 1933)

Klein, J. Raymond, Ph.D. University of Illinois, Neuropsychiatric Institute, 912 S. Wood St., Chicago. *Biochemist and Assistant Professor of Psychiatry and Physiological Chemistry.* (2, 1941)

Kleiner, Israel Simon, Ph.D. New York Medical College, Flower and Fifth Avenue Hospitals, New York City. *Professor of Physiology and Biochemistry.* (1, 1911; 2, 1912; 3, 1912; 5, 1933)

Kleitman, Nathaniel, A.M., Ph.D. University of Chicago, Chicago, Ill. *Associate Professor of Physiology.* (1, 1923)

Klemperer, Friedrich Wilhelm, M.D. Harvard Medical School, Boston, Mass. *Assistant in Medicine.* (2, 1941)

Kletzien, Seymour W., M.S., Ph.D. State Institute for the Study of Malignant Disease, 113 High St., Buffalo, N. Y. *Biochemist.* (5, 1933)

Kline, O. L., Ph.D. U. S. Department of Agriculture, Food and Drug Administration, Washington, D. C. *Biochemist.* (5, 1936)

Klüver, Heinrich, Ph.D. University of Chicago, Chicago, Ill. *Member, Otho S. A. Sprague Memorial Institute.* (1, 1935)

Knoefel, Peter K., M.A., M.D. University of Louisville, Louisville, Ky. *Professor of Pharmacology.* (3, 1934)

Knowlton, Frank P., A.M., M.D. Syracuse University College of Medicine, Syracuse, N. Y. *Professor of Physiology.* (1, 1911)

Knowlton, G. Clinton, Ph.D. University of Iowa, Iowa City. *Assistant Professor of Physiology.* (1, 1938)

Knudson, Arthur, Ph.D. Albany Medical College, Albany, N. Y. *Professor of Biochemistry, Medical Department of Union University.* (2, 1919; 5, 1936)

Knutti, Ralph Eddy, M.D. University of Rochester School of Medicine and Dentistry, 260 Crittenden Blvd., Rochester, N. Y. *Assistant Professor of Pathology.* (4, 1933)

Kober, Philip A. Sherman Laboratories, Detroit, Mich. *Director of Research.* (2, 1912)

Koch, Elizabeth M., M.A., Ph.D. 1534 E. 59th St., Chicago, Ill. (2, 1925)

Koch, Fred Conrad, M.S., Ph.D. 1534 E. 59th St., Chicago, Ill. *Frank P. Hixon Distinguished Service Professor Emeritus of Biochemistry, University of Chicago; Research Biochemist, Armour and Co.* (2, 1912; 5, 1933)

Kochakian, Charles D., A.M., Ph.D. University of Rochester Medical School, 260 Crittenden Blvd., Rochester, N. Y. *Research Associate, Dept. of Vital Economics.* (1, 1942)

Kocher, Rudolph Alfred, M.D. Box 926, Carmel, Calif. *Director, Velie Metabolic Clinic.* (2, 1915)

Koehler, Alfred E., M.D., Ph.D. 317 W. Pueblo St., Santa Barbara, Calif. *Physician, Sansum Clinic, Santa Barbara Cottage Hospital.* (2, 1924)

Koehne, Martha, Ph.D. Ohio State Department of Health, 75 Eighteenth Ave., Columbus. *Nutritionist.* (5, 1933)

Koepf, George F., M.D. Buffalo General Hospital, 100 High St., Buffalo, N. Y. *Instructor in Medicine, University of Buffalo.* **(1,** 1942)

Kohn, Henry I., Ph.D. Duke University School of Medicine, Durham, N. C. *Assistant Professor of Physiology and Pharmacology.* **(1,** 1940)

Kolmer, John A., M.S., M.D., D.P.H., Sc.D., LL.D., L.H.D. 1 Montgomery Ave., Bala-Cynwyd, Pa. *Professor of Medicine, Temple University; Director, Research Institute of Cutaneous Medicine.* **(6,** 1913)

Komarov, Simon A., M.S., M.D., Ph.D. McGill University, Montreal, Quebec, Canada. *Lecturer in Physiology.* **(1,** 1933)

Kopeloff, Nicholas, Ph.D. New York State Psychiatric Institute, 722 W. 168th St., New York City. *Principal Research Bacteriologist, New York State Psychiatric Institute and Hospital.* **(6,** 1937)

Koppanyi, Theodore, Ph.D. Georgetown University, Washington, D. C. *Professor of Pharmacology.* **(1,** 1924; **3,** 1935)

Korr, Irwin M., M.A., Ph.D. New York University College of Medicine, 477 First Ave., New York City. *Instructor in Physiology.* **(1,** 1939)

Kozelka, Frank L., Ph.D. Dept. of Pharmacology and Toxicology, University of Wisconsin, Madison. *Assistant Professor of Toxicology.* **(3,** 1939)

Krahl, Maurice E., Ph.D. Lilly Research Laboratories, Eli Lilly & Co., Indianapolis, Ind. *Research Biological Chemist.* **(2,** 1939)

Kramer, Benjamin, A.M., M.D. 6 Pierrepont St., Brooklyn, N. Y. *Pediatrician-in-Chief, Brooklyn Jewish Hospital; Professor of Clinical Pediatrics, Long Island College Medical School.* **(1,** 1915; **2,** 1914)

Kramer, Martha, Ph.D. Department of Home Economics, Yenching University, Peiping, China. *Professor of Food Economics and Nutrition.* **(5,** 1933)

Krantz, John C., Jr., M.S., Ph.D. University of Maryland Medical School, Baltimore. *Professor of Pharmacology.* **(3,** 1937)

Krauss, William E., Ph.D. Ohio Experiment Station, Wooster. *Chief, Dairy Department.* **(2,** 1932; **5,** 1933)

Kraybill, Henry R., M.S., Ph.D. 5720 Woodlawn Ave., Chicago, Ill. *Professorial Lecturer, Department of Biochemistry, University of Chicago; Director, Department of Scientific Research, American Meat Institute.* **(2,** 1942)

Krayer, Otto, M.D. Harvard Medical School, 25 Shattuck St., Boston, Mass. *Associate Professor of Comparative Pharmacology.* **(3,** 1938)

Krueger, Albert Paul, M.D. University of California, Berkeley. *Professor of Bacteriology; Commander M.C. U.S.N.R. Officer in charge Lab. Research Unit no. 1, Berkeley, Calif.* **(4,** 1930; **6,** 1937)

Krueger, Hugo M., Ph.D. St. Louis University Medical School, 1402 S. Grand Blvd., St. Louis, Mo. *Assistant Professor of Pharmacology.* **(1,** 1931; **3,** 1935)

Krumbhaar, Edward B., M.D., Ph.D. University of Pennsylvania Medical School, Philadelphia. *Professor of Pathology.* **(1,** 1914; **4,** prior to 1920)

Kruse, Harry Dayton, M.D., Sc.D. Milbank-Memorial Fund, 40 Wall St., New York City. **(2,** 1933)

Kruse, Theophile K., A.M., Ph.D. University of Pittsburgh Medical School, Pittsburgh, Pa. *Professor of Physiology and Pharmacology.* **(1,** 1919; **3,** 1920)

Kubie, Lawrence S., M.D. 7 E. 81st St., New York City. *Associate in Neurology, College of Physicians and Surgeons, Columbia University; Associate Psychiatrist, Mt. Sinai Hospital, New York City.* **(4,** 1928)

Kuhn, Harry A., M.S. 45 Broadway, New York City. *Executive Officer, C. W. Procurement District.* **(3,** 1927)

Kuhn, Ludwig R., Ph.D. Department of Bacteriology, University of Georgia, Athens. *Associate Professor of Bacteriology and Immunology.* **(6,** 1939)

Kunde, Margarete M., Ph.D., M.D. 116 S. Michigan Ave., Chicago, Ill. *Instructor in Medicine, Northwestern University Medical School.* **(1,** 1924)

Kurtz, Alton C., Ph.D. Department of Biochemistry, Medical School, University of Oklahoma, Oklahoma City. *Assistant Professor.* **(2,** 1942)

Kydd, David M., M.D. Mary Imogene Bassett Hospital, Cooperstown, N. Y. *Associate Physician.* **(5,** 1934)

Kyes, Preston, A.M., Sc.D., M.D. North Jay, Me. **(6,** 1918)

Lacy, G. R., M.D. University of Pittsburgh, Pittsburgh, Pa. *Professor of Bacteriology and Immunology.* **(4,** 1927)

Lamb, Alvin R., M.S., Ph.D. Experiment Station, Hawaiian Sugar Planters' Association, Honolulu. *Research Associate.* **(2,** 1923; **5,** 1934)

Lambert, Robert A., M.D. Rockefeller Foundation, 49 W. 49th St., New York City. *Associate Director for the Medical Sciences.* **(4,** 1922)

Lamson, Paul Dudley, M.D. Vanderbilt University Medical School, Nashville, Tenn. *Professor of Pharmacology.* **(1,** 1921; **3,** 1915)

Lamson, Robert W., A.M., Ph.D., M.D. Suite 810, 1930 Wilshire Blvd., Los Angeles, Calif. *Professor of Medicine and Public Health, Uni-*

versity of Southern California School of Medicine. (6, 1928)

Lancefield, Rebecca C., Ph.D. 4 Kenmore Rd., Douglaston, Long Island, N. Y. *Associate Member, Rockefeller Institute for Medical Research.* (6, 1933)

Landis, Carney, Ph.D. Psychiatric Institute and Hospital, Columbia University, 722 W. 168th St., New York City. *Principal Research Psychologist and Associate Professor of Psychology.* (1, 1939)

Landis, Eugene Markley, Ph.D., M.D. University of Virginia School of Medicine, University. *Professor of Internal Medicine.* (1, 1928)

Lands, Alonzo M., M.A., Ph.D. Frederick Stearns and Co., 6533 Jefferson Ave., Detroit, Mich. *Research Assistant, Dept. of Physiology and Pharmacol.* (1, 1942)

Landsteiner, Karl, M.D. Rockefeller Institute for Medical Research, 66th St. and York Ave., New York City. *Member, Rockefeller Institute for Medical Research, Member National Academy of Sciences.* (4, 1925; 6, 1922)

Lange, Carl, M.D. 371 Morris St., Albany, N. Y. *Associate Bacteriologist, Divisions of Laboratories and Public Health, New York State Department of Health.* (6, 1938)

Langley, Wilson D., Ph.D. University of Buffalo Medical School, Buffalo, N. Y. *Associate Professor of Biological Chemistry.* (2, 1937)

Langworthy, Orthello R., M.A., M.D. Johns Hopkins Hospital, Baltimore, Md. *Associate Professor of Neurology, Johns Hopkins University.* (1, 1928)

Larrabee, Martin G., Ph.D. Johnson Foundation, Hospital of University of Pennsylvania, Philadelphia. *Fellow in Medical Physics and Lecturer in Biophysics.* (1, 1940)

Larson, Edward, Ph.D. Temple University Medical School, Broad and Ontario Sts., Philadelphia, Pa. *Associate Professor of Pharmacology.* (1, 1939; 3, 1937)

Larson, Hardy W., A.M., Ph.D. Metropolitan Life Insurance Co., Biochemical Laboratory, 1 Madison Ave., New York City. *Research Chemist.* (2, 1937)

Larson, Paul S., Ph.D. Medical College of Virginia, Richmond. *Associate in Physiology and Pharmacology.* (1, 1939)

Larson, W. P., M.D. University of Minnesota, Minneapolis. *Professor and Head of Department of Bacteriology and Immunology.* (6, 1917)

Lashley, Karl S., M.S., Ph.D. The Biological Laboratories, Harvard University, Cambridge, Mass. *Research Professor of Neuropsychology; Member of the National Academy of Sciences.* (1, 1923)

Laug, E. P., M.A., Ph.D. Division of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D. C. *Pharmacologist.* (2, 1938)

Laurens, Henry, A.M., Ph.D., LL.D. School of Medicine, Tulane University, Station 20, New Orleans, La. *Professor of Physiology.* (1, 1913)

Lavine, T. F., Ph.D. Lankenau Hospital Research Institute, Philadelphia, Pa. *Research Chemist.* (2, 1938)

Lawson, Hampden, M.D., Ph.D. University of Louisville, Louisville, Ky. *Professor of Physiology.* (1, 1933)

Leake, Chauncey D., M.S., Ph.D. The University of Texas Medical Branch, Galveston. *Vice-President of the University of Texas in Charge of the Medical Program.* (1, 1923; 3, 1924)

Leathes, John Beresford, M.A., M.B., F.R.C.S., F.R.S. 106 Banbury Rd., Oxford, England. (2, 1909)

Lederer, Ludwig George, Ph.D., M.D. Pennsylvania Central Airlines, National Airport, Washington, D. C. *Assistant Chief, Medical Department.* (1, 1940)

Lederer, Max, M.D. 1037 President St., Brooklyn, N. Y. *Director of Laboratories, Jewish Hospital.* (6, 1920)

Lee, Milton O., M.A., Ph.D. Harvard Medical School, Boston, Mass. *Associate, Memorial Foundation for Neuro-endocrine Research; Research Fellow in Physiology.* (1, 1927; 5, 1933)

Lee, Robert C., B.Ch.E. Nutrition Laboratory, 29 Vila St., Boston, Mass. (5, 1940)

Leese, Chester E., M.S., Ph.D. George Washington University School of Medicine, Washington, D. C. *Associate Professor of Physiology.* (1, 1934)

Lehman, Arnold J., Ph.D., M.D. Wayne University College of Medicine, Detroit, Mich. *Associate Professor of Pharmacology.* (3, 1937)

Lehman, Robert A., M.S., Ph.D. New York University College of Medicine, 477 First Ave., New York City. *Instructor in Therapeutics.* (3, 1942)

Lehmann, Gerhard, M.D., Dr. Ing. University of Louisville School of Medicine, Louisville, Ky. *Associate Professor of Pharmacology.* (3, 1939)

Lenhart, Carl H., M.D. Lakeside Hospital, 2065 Adelbert Rd., Cleveland, O. *Oliver H. Payne Professor of Surgery, Western Reserve University.* (1, 1921)

Lennette, Edwin H., Ph.D., M.D. Caixa Postal 49, Rio de Janeiro, Brazil. *Staff Member, International Health Division, The Rockefeller Foundation.* (4, 1941)

Leonard, Clifford Shattuck, M.S., Ph.D. University of Vermont Medical College, Burlington. *Assistant Professor of Pharmacology.* (3, 1927)

Lepkovsky, Samuel, M.S., Ph.D. University of California, Berkeley. *Associate Professor of Poultry Husbandry.* (2, 1933; 5, 1933)

L'Esperance, Elise L., M.D. 321 E. 15th St., New York City. *Director of Laboratories, New York Infirmary for Women and Children.* (6, 1920)

Leverton, Ruth M., Ph.D. Department of Home Economics, University of Nebraska, Lincoln. *Associate Professor Human Nutrition Research.* (5, 1942)

Levin, Isaac, M.D. 57 W. Fifty-seventh St., New York City. *Clinical Professor of Cancer Research, New York University; Chief of the Department of Cancer Service, Montefiore Hospital; Director, New York City Cancer Institute.* (1, 1900)

Levin, Louis, Ph.D. Department of Anatomy, College of Physicians and Surgeons, Columbia University, New York City. *Research Associate in Anatomy.* (2, 1939)

Levine, Harold, Ph.D. Pabst Brewing Co., 917 W. Juneau Ave., Milwaukee, Wis. *Biochemist.* (2, 1933; 5, 1933)

Levine, Milton, M.S., Ph.D. 225a Millard Hall, University of Minnesota, Minneapolis. *Bacteriologist, University Hospital; Instructor, Medical School.* (6, 1942)

Levine, Philip, M.D., M.A. Newark Beth Israel Hospital, 201 Lyons Ave., Newark, N. J. *Serologist and Bacteriologist.* (6, 1925)

Levine, Rachmiel, M.D., C.M. Michael Reese Hospital, Chicago, Ill. *Assistant Director, Dept. of Metabolism.* (1, 1942)

Levine, Samuel Z., M.D., New York Hospital, 525 E. 68th St., New York City. *Professor of Pediatrics, Cornell University Medical College; Pediatrician-in-Chief, New York Hospital.* (5, 1933)

Levine, Victor Emanuel, A.M., Ph.D., M.D. Creighton University School of Medicine, Omaha, Neb. *Professor of Biological Chemistry and Nutrition.* (2, 1936)

Levinson, Samuel A., M.D. University of Illinois College of Medicine, 808 S. Wood St., Chicago. *Professor of Pathology.* (4, 1938)

Levison, Louis A., M.D. 421 Michigan St., Toledo, O. *Physician to Toledo Hospital; Physician to St. Vincent Hospital.* (6, 1916)

Levy, Milton, Ph.D. 477 First Ave., New York City. *Assistant Professor of Chemistry, New York University College of Medicine.* (2, 1933)

Levy, Robert L., M.D. 730 Park Ave., New York City. *Professor of Clinical Medicine, College of Physicians and Surgeons, Columbia University.* (3, 1915)

Lewey, F. H., M.D. University Hospital, University of Pennsylvania, Philadelphia. *Visiting Professor of Neurophysiology and Consultant in Neurology.* (1, 1937)

Lewis, Howard Bishop, Ph.D. Medical School, University of Michigan, Ann Arbor. *Professor of Biological Chemistry and Director of the College of Pharmacy.* (1, 1925; 2, 1913; 5, 1933)

Lewis, Julian Herman, M.D. 4750 Champlain Ave., Chicago, Ill. *Associate Professor of Pathology, University of Chicago; Member of the Otho S. A. Sprague Memorial Institute.* (4, 1924)

Lewis, Robert C., Ph.D. 4200 E. 9th Ave., Denver, Colo. *Professor of Biochemistry, School of Medicine, University of Colorado.* (2, 1931; 5, 1933)

Lewis, Warren H., M.D. The Wistar Institute of Anatomy and Biology, Woodland Ave. and 36th St., Philadelphia, Pa. *Member; Member of the National Academy of Sciences.* (1, 1919)

Li, Richard D., M.D. Peiping Union Medical College, Peiping, China. *Instructor in Pharmacology.* (3, 1941)

Libby, Raymond L., M.S., Ph.D. American Cyanamid Co., 1937 W. Main St., Stamford, Conn. *Bio-physicist.* (6, 1938)

Libet, Benjamin, Ph.D. 111 N. 49th St., Philadelphia, Pa. *Research Associate, Institute of Pennsylvania Hospital, Philadelphia.* (1, 1942)

Libman, Emanuel, M.D. 180 E. 64th St., New York City. *Consulting Physician, Mount Sinai Hospital.* (6, 1920)

Liddell, Howard S., A.M., Ph.D. Cornell University, Ithaca, N. Y. *Professor of Psychology.* (1, 1925)

Lieb, Charles C., M.D. 630 W. 168th St., New York City. *Hosack Professor of Pharmacology, College of Physicians and Surgeons, Columbia University.* (1, 1936; 3, 1915)

Lieberman, Arnold L., M.D., Ph.D. 738 Broadway, Suite 502, Gary, Ind. *Assistant in Medicine, Northwestern University.* (1, 1934)

Lightbody, Howard D., M.S., Ph.D. Western Regional Research Laboratory, U. S. Department of Agriculture, Albany, Calif. *Principal Biochemist.* (2, 1936)

Lillie, Ralph Stayner, Ph.D., Sc.D. University of Chicago, Chicago, Ill. *Professor Emeritus of General Physiology; Physiologist, Marine Biological Laboratory, Woods Hole, Mass.* (1, 1905; 2, 1913)

Lillie, R. D., M.D., U. S. Public Health Service, National Institute of Health, Bethesda, Md. *Senior Surgeon.* (4, 1941)

Lim, Robert Kho-Seng, Ph.D., D.Sc., F.R.S.E. Peiping Union Medical College, Peiping, China. *Professor of Physiology, Director of Medical Relief Corps, China.* (1, 1923)

Lindsley, Donald B., M.A., Ph.D. Bradley Home, East Providence, R.I. *Director of Psychological and Neurophysiological Laboratory; Assistant Professor of Psychology, Brown University.* (1, 1937)

Linegar, Charles R., Ph.D. Georgetown University School of Medicine, Washington, D. C. *Associate Professor of Pharmacology.* (3, 1938)

Lineweaver, Hans, M.A., Ph.D. Western Regional Research Laboratory, U. S. Department of Agriculture, Albany, Calif. *Senior Biochemist.* (2, 1941)

Link, Karl Paul, Ph.D. Biochemistry Building, University of Wisconsin, Madison. *Professor of Biochemistry.* (2, 1931)

Lintz, William, M.D. 36 Plaza St., Brooklyn, N. Y. *Late Professor of Immunology and Bacteriology and Clinical Professor of Medicine, Long Island College of Medicine.* (6, 1920)

Lipman, Mrs. Miriam O., A.M. Presbyterian Hospital, 620 W. 168th St., New York City. *Research Assistant, Edward Daniels Faulkner Arthritis Clinic.* (6, 1931)

Lipmann, Fritz, M.D., Ph.D. Biochemical Research Laboratory, Massachusetts General Hospital, Boston. *Research Chemist.* (2, 1941)

Litchfield, John T., Jr., M.D. Johns Hopkins School of Medicine, Baltimore, Md. *Associate in Pharmacology and Experimental Therapeutics.* (3, 1940)

Little, James Maxwell, M.S., Ph.D. Bowman Gray School of Medicine of Wake Forest College, Winston-Salem, N. C. *Assistant Professor of Physiology and Pharmacology.* (1, 1942)

Livingston, Alfred E., Ph.D. Temple University School of Medicine, Philadelphia, Pa. *Professor of Pharmacology.* (1, 1917; 3, 1920)

Lloyd, David P. C., D.Ph. The Rockefeller Institute for Medical Research, 66th St. and York Ave., New York City. *Associate in Physiology.* (1, 1939)

Locke, Arthur P., Ph.D. Institute of Pathology, Western Pennsylvania Hospital, Pittsburgh. *Research Biochemist.* (6, 1926)

Lodholz, Edward, M.D. Medical Laboratories, University of Pennsylvania, Philadelphia. *Isaac Ott Professor of Physiology, Graduate School of Medicine.* (1, 1913)

Loeb, Leo, M.D. Washington University Medical School, St. Louis, Mo. *Professor Emeritus of Pathology.* (1, 1907; 4, prior to 1920)

Loebel, Robert O., M.D. Russell Sate Institute of Pathology, Cornell Medical College, 1300 York Ave., New York City. *Research Fellow; Adjunct Assistant Visiting Physician, Second (Cornell) Medical Division of Bellevue Hospital.* (1, 1928)

Loew, Earl R., M.S., Ph.D. Parke, Davis & Co., Detroit, Mich. (1, 1940)

Loewe, S., M.D. 17 Cole Terrace, New Rochelle, N. Y. *Hon. Prof. Pharmacology, Heidelberg; Dept. of Pharmacology, Cornell University Medical College.* (3, 1936)

Logan, Milan A., Ph.D. University of Cincinnati School of Medicine, Cincinnati, O. *Professor of Biological Chemistry.* (2, 1936)

Long, C. N. H., M.Sc., D.Sc., M.D. Yale University, New Haven, Conn. *Sterling Professor of Physiological Chemistry.* (1, 1935; 2, 1927)

Long, Esmond R., M.D. 7th and Lombard Sts., Philadelphia, Pa. *Director, Henry Phipps Institute; Professor of Pathology, University of Pennsylvania.* (4, 1930)

Long, Perrin Hamilton, M.D. The Johns Hopkins University, 615 N. Wolfe St., Baltimore, Md. *Professor of Preventive Medicine.* (3, 1940)

Longcope, Warfield T., M.D. Johns Hopkins Hospital, Baltimore, Md. *Professor of Medicine, Johns Hopkins University.* (3, 1921; 4, prior to 1920; 6, 1923)

Longenecker, Herbert Eugene, M.S., Ph.D. Department of Chemistry, University of Pittsburgh, Pittsburgh, Pa. *Assistant Professor of Chemistry and Associate Director of Research.* (2, 1940)

Looney, Joseph Michael, M.D. 728 Pleasant St., Worcester, Mass. *Major, U. S. Army.* (2, 1922)

Loosli, Clayton Garr, M.D. The University of Chicago, Department of Medicine, Chicago, Ill. *Assistant Professor.* (4, 1940)

Lorente de Nó, Rafael, M.D. The Rockefeller Institute for Medical Research, 66th St. and York Ave., New York City. *Member.* (1, 1937)

Lorenz, Egon, Ph.D. National Cancer Institute, Bethesda, Md. *Senior Biophysicist.* (4, 1942)

Loring, H. S., M.S., Ph.D. Stanford University, Calif. *Assistant Professor of Biochemistry.* (2, 1938)

Lothrop, Alfred P., M.A., Ph.D. 279 Elm St., Oberlin, O. *Professor of Organic Chemistry, Oberlin College.* (2, 1912)

Loveless, Mary H., M.D. New York Hospital, 525 E. 68th St., New York City. *Research Associate, Cornell Medical School; Physician to Out-Patients, New York Hospital.* (6, 1941)

Lowell, Francis C., M.D. Nine Acre Corner, Concord, Mass. *Instructor in Medicine, Boston City Hospital.* (6, 1942)

Lowry, Oliver H., M.D., Ph.D. Research Laboratory, Public Health Research Institute of the City of New York, Foot of E. 15th St. *Research Associate.* (2, 1942)

Lubinski, Herbert, M.D. Jewish General Hospital, 3755 St. Catherine Rd., Montreal, Canada. *Bacteriologist.* (6, 1941)

Lucas, George H. W., M.A., Ph.D. University of Toronto, Toronto, Canada. *Associate Professor of Pharmacology.* (2, 1925; 3, 1928)

Luck, James Murray, Ph.D. Stanford University, Stanford, Calif. *Professor of Biochemistry.* (2, 1925)

Lucké, Balduin, M.D. 141 Montgomery Ave., Bala-Cynwyd, Pa. *Professor of Pathology, University of Pennsylvania Medical School.* (4, 1924)

Luckhardt, Arno Benedict, M.S., Ph.D., M.D. University of Chicago, Chicago, Ill. *Professor of Physiology.* (1, 1911)

Ludewig, Stephan, Ph.D. University of Virginia School of Medicine, University. *Assistant Professor of Biochemistry.* (2, 1941)

Luduena, Froilan P., Ph.D., M.D. Faculty of Medicine, Rosario, Argentina. *Professor Adjunto de Farmacologia.* (3, 1941)

Lukens, Francis D. W., M.D. University of Pennsylvania, 809 Maloney Clinic, 36th and Spruce Sts., Philadelphia. *Assistant Professor of Medicine and Director, George S. Cox Medical Research Institute.* (1, 1938)

Lund, E. J., Ph.D. Department of Zoology and Physiology, University of Texas, Austin. *Professor of General Physiology.* (1, 1930)

Lundgren, Harold P., Ph.D. Western Regional Research Laboratory, U.S.D.A., Albany, Calif. *Chemist.* (2, 1942)

Lundy, John Silas, M.D. The Mayo Foundation, Rochester, Minn. *Chief of Section on Anesthesia.* (3, 1935)

Lurie, Max B., M.D. Henry Phipps Institute, 7th and Lombard Sts., Philadelphia, Pa. *Assistant Professor of Experimental Pathology.* (4, 1934; 6, 1930)

Lutz, Brenton R., Ph.D. Boston University, 688 Boylston St., Boston, Mass. *Professor of Biology.* (1, 1925)

Luyet, Basile J., Sc.D. (Biol.), Sc.D. (Physics). St. Louis University School of Medicine, St. Louis, Mo. *Professor of Biology.* (1, 1936)

Lyall, Harold W., A.M., Ph.D. Division of Laboratories and Research, New York State Department of Health, Albany. *Assistant Director in charge of Antitoxin, Serum, and Vaccine Laboratories.* (6, 1937)

Lyman, Carl M., Ph.D. Division of Swine Husbandry, Agricultural Experiment Station, College Station, Texas. *Nutritionist.* (2, 1940)

Lyman, John F., Ph.D. Townshend Hall, Ohio State University, Columbus. *Professor of Agricultural Chemistry.* (2, 1920; 5, 1933)

Macallum, A. Bruce, M.D., Ph.D. Medical School, University of Western Ontario, London, Ont., Canada. *Professor of Biochemistry.* (2, 1914)

MacArthur, Edith H., A.M., Ph.D. Skidmore College, Saratoga Springs, N. Y. *Professor and Director of Home Economics.* (5, 1933)

MacCorquodale, D. W., M.S., Ph.D. Abbott Laboratories, North Chicago, Ill. *Head, Biochemical Research.* (2, 1934)

MacFadyen, Douglas A., M.A., M.D. Alfred I. duPont Institute, Wilmington, Del. *Chief of Biochemistry.* (2, 1942)

MacKay, Eaton M., M.D. The Scripps Metabolic Clinic, La Jolla, Calif. (1, 1930)

Mackenzie, Cosmo G., D.Sc. The Johns Hopkins University, Baltimore, Md. *Associate in Biochemistry, School of Hygiene and Public Health.* (5, 1942)

Mackenzie, George M., M.D. Mary Imogene Bassett Hospital, Cooperstown, N. Y. *Physician-in-Chief; Director, Otsego County Laboratories.* (6, 1921)

MacLeod, Colin M., M.D. New York University College of Medicine, 477 First Ave., New York City. *Professor of Bacteriology.* (6, 1937)

MacLeod, Florence L., M.A., Ph.D. University of Tennessee, Knoxville. *Professor of Nutrition.* (2, 1927; 5, 1933)

MacLeod, Grace, M.A., Ph.D. 106 Morningside Drive, New York City. *Professor of Nutrition, Teachers College, Columbia University.* (2, 1924; 5, 1933)

MacLeod, John, M.S., Ph.D. Cornell University Medical College, 1300 York Ave., New York City. *Research Associate of Anatomy.* (1, 1942)

MacNeal, Ward J., M.D. New York Post-Graduate Medical School and Hospital, 303 E. 20th St., New York City. *Professor of Bacteriology.* (4, 1925)

MacNider, William deB., M.D., Sc.D., LL.D. University of North Carolina, Chapel Hill. *Kenan Research Professor of Pharmacology; Member, National Academy of Sciences.* (1, 1912; 2, 1912; 3, 1909; 4, prior to 1920)

Macht, David Israel, M.D., Phar. D. (hon.), Litt. D. Charles and Chase Sts., Baltimore, Md. *Director of Pharmacological Research Laboratory, Hynson, Westcott and Dunning, Inc.; Professorial Lecturer in Physiology, Yeshiva College, New York City.* (1, 1916)

Madden, Sidney C., M.D. University of Rochester School of Medicine and Dentistry, Rochester, N. Y. *Assistant Professor of Pathology.* (4, 1939)

Maddock, Stephen, M.D. Boston City Hospital, Boston, Mass. *Assistant in Surgery, Harvard Medical School; Director of Surgical Research Laboratory, City Hospital.* (4, 1931)

Madsen, Louis L., Ph.D. Bureau of Animal Industry, U. S. Department of Agriculture, Box 71, Berwyn, Md. *Nutritionist.* (5, 1940)

Magath, Thomas B., M.S., Ph.D., M.D. Mayo Clinic, Rochester, Minn. *Associate Professor of Clinical Bacteriology and Parasitology, University of Minnesota, Mayo Foundation; Consultant Physician in Clinical Laboratories, Mayo Clinic.* (1, 1928)

Magill, Thomas P., M.D. Cornell University Medical College, 1300 York Ave., New York City. (6, 1937)

Magoun, Horace W., Ph.D. Northwestern University Medical School, 303 E. Chicago Ave., Chicago, Ill. *Associate Professor of Neurology.* (1, 1937)

Mahon, Eleanor Conway, Ph.D. Iron River, Mich. (4, 1940)

Main, Rolland J., Ph.D. Medical College of Virginia, Richmond. *Professor of Physiology.* (1, 1936)

Maison, George L., M.S., M.D. Aeromedical Research Laboratory, Wright Field, Dayton, O. *Second Lieutenant, Air Corps, U. S. Army; Assistant Professor of Physiology, Wayne University, Detroit, Michigan.* (1, 1939)

Major, Randolph T., M.Sc., Ph.D. Cole Ave., Mountainside, Westfield, N. J. *Director of Research, Merck & Co.* (2, 1942)

Mallory, G. Kenneth, M.D. Mallory Institute of Pathology, Boston City Hospital, Boston, Mass. *Associate Professor.* (4, 1940)

Mallory, Tracy B., M.D. Massachusetts General Hospital, Boston. *Chief of Pathology and Bacteriology; Assistant Professor of Pathology, Harvard Medical School.* (4, 1937)

Maloney, Arnold H., Ph.D., M.D., LL.D. Howard University School of Medicine, Washington, D. C. *Professor and Head of Department of Pharmacology.* (3, 1932)

Maltaner, Frank, Ph.D. 388 New Scotland Ave., Albany, N. Y. *Associate Biochemist, Division of Laboratories and Research, New York State Department of Health.* (6, 1920)

Maluf, N. S. Rustum, M.S., Ph.D. Georgetown University Medical School, Washington, D. C. *Instructor in Physiology.* (1, 1942)

Man, Evelyn B., Ph.D. Department of Psychiatry and Mental Hygiene, Yale University School of Medicine, New Haven, Conn. *Assistant Professor in the Biochemistry Laboratory.* (2, 1936)

Manery, Jeanne Forest, M.A., Ph.D. Medical School, University of Toronto, Toronto, Ont., Canada. *Demonstrator in Biochemistry.* (1, 1937)

Mann, Frank C., M.A., M.D., Sc.D., LL.D. Mayo Clinic, Rochester, Minn. *Director, Division of Experimental Medicine; Professor of Experimental Medicine, Mayo Foundation.* (1, 1916; 3, 1923; 4, 1924)

Manville, Ira Albert, M.A., M.D., Ph.D. University of Oregon Medical School, Portland. *Associate Clinical Professor of Medicine and Director of Nutritional Research Laboratories.* (1, 1933)

Manwaring, Wilfred H., M.D. Stanford University, Palo Alto, Calif. *Professor Emeritus of Bacteriology and Experimental Pathology.* (4, prior to 1920; 6, 1917)

Marine, David, A.M., M.D. Montefiore Hospital, Gunhill Road and East 210th St., New York City. *Director of Laboratories.* (1, 1910; 4, prior to 1920)

Markowitz, J., M.D., Ph.D. 220 Bloor St., Toronto, Ont., Canada. *Research Associate in Physiology, University of Toronto, Faculty of Medicine.* (1, 1929)

Marmont, George H., Ph.D. Columbia University, 630 W. 168th St., New York City. *Research Associate in Physiology.* (1, 1941)

Marrazzi, Amedeo S., M.D., 477 First Ave., New York City. *Assistant Professor of Pharmacology, New York University College of Medicine.* (3, 1938)

Marsh, M. Elizabeth, M.S., Ph.D. Killian Research Laboratories, 49 W. 45th St., New York City. *Assistant Director.* (1, 1929; 5, 1933)

Marshak, Alfred George, M.A., Ph.D. Radiation Laboratory, University of California, Berkeley. *Research Associate and Finney-Howell Fellow.* (1, 1940)

Marshall, Eli Kennerly, Jr., Ph.D., M.D., LL.D. Johns Hopkins Medical School, Baltimore, Md. *Professor of Pharmacology and Experimental Therapeutics.* (1, 1915; 2, 1913; 3, 1915)

Marshall, Wade H., Ph.D. Wilmer Ophthalmological Institute, Johns Hopkins Hospital, Baltimore, Md. *Associate in Physiological Optics, Johns Hopkins University.* (1, 1937)

Martin, Donald Stover, M.D. Duke University School of Medicine, Duke Hospital, Durham, N. C. *Associate Professor of Bacteriology.* (4, 1940)

Martin, Stevens J., M.A., Ph.D. Tilton General Hospital, Fort Dix, N. J. *Capt. M. C., Chief of Sections on Anesthesia and Operating Pavilion, and Resuscitation and Oxygen Therapy.* (1, 1933)

Mason, Edward C., M.D., Ph.D. University of Oklahoma School of Medicine, Oklahoma City. *Professor of Physiology.* (1, 1935)

Mason, H. L., M.A., Ph.D. Mayo Clinic, Rochester, Minn. *Associate Professor of Physiological Chemistry, The Mayo Foundation, University of Minnesota.* (2, 1941)

Mason, Karl Ernest, Ph.D. The University of Rochester, School of Medicine and Dentistry, Rochester, N. Y. *Professor of Anatomy.* (1, 1932; 5, 1941)

Mason, Morton F., Ph.D. Vanderbilt University Medical School, Nashville, Tenn. *Associate Professor of Biochemistry; Research Associate in Medicine.* (2, 1938)

Massengale, Oliver N., Ph.D. Mead Johnson & Co., Research Laboratory, Evansville, Ind. *Research Biochemist.* (2, 1937)

Mast, S. O., Ph.D. Johns Hopkins University, Baltimore, Md. *Professor of Zoology.* (1, 1920)

Mathews, Albert P., Ph.D., D.Sc. (hon.). Woods Hole, Mass. *Professor of Biochemistry Emeritus, University of Cincinnati.* (1, 1898; 2, 1906)

Mattill, Henry A., A.M., Ph.D. State University of Iowa, Iowa City. *Professor of Biochemistry.* (1, 1913; 2, 1909; 5, 1933)

Maurer, Frank W., Ph.D. Harvard School of Public Health, 55 Shattuck St., Boston, Mass. *Assistant Professor of Physiology.* (1, 1941)

Mavor, James Watt, Ph.D. Union College, Schenectady, N. Y. *Professor of Biology.* (1, 1930)

Mayerson, Hymen S., Ph.D. Tulane University School of Medicine, Station 20, New Orleans, La. *Associate Professor of Physiology.* (1, 1928)

Maynard, Leonard A., Ph.D. Cornell University, Ithaca, N. Y. *Professor of Animal Nutrition; Director of United States Soil, Plant and Nutrition Laboratory; Laboratory of Animal Nutrition.* (2, 1930; 5, 1933)

McCann, William S., M.D., D.Sc. (hon.). University of Rochester, School of Medicine, Rochester, N. Y. *Dewey Professor of Medicine.* (2, 1923; 5, 1933)

McCarrell, June D., M.A., Ph.D. Dept. Surgical Research, Massachusetts General Hospital, Boston. *Research Fellow in Anesthesia, Massachusetts General Hospital; Instructor in Physiology, Harvard School of Public Health.* (1, 1942)

McCay, Clive M., M.S., Ph.D. Animal Nutrition Laboratory, Cornell University, Ithaca, N. Y. *Professor of Animal Nutrition.* (2, 1929; 5, 1933)

McClellan, Walter S., M.D. Saratoga Spa, Saratoga Springs, N. Y. *Medical Director; Associate Professor of Medicine, Albany Medical College.* (1, 1931)

McClendon, J. F., M.S., Ph.D. Hahnemann Medical College, 235 N. 15th St., Philadelphia, Pa. *Research Professor in Physiology.* (1, 1910; 2, 1914; 5, 1935)

McClosky, William T., B.A. 5120 7th St., N.W., Washington, D. C. *Senior Pharmacologist, Div. of Pharmacology, Federal Security Agency, U. S. Department of Agriculture.* (3, 1929)

McCollum, Elmer Verner, M.A., Ph.D., Sc.D., LL.D. Johns Hopkins University, School of Hygiene and Public Health, 615 N. Wolfe St., Baltimore, Md. *Professor of Biochemistry; Member National Academy of Sciences.* (2, 1910; 5, 1933)

McCouch, Grayson Prevost, M.D. University of Pennsylvania, Philadelphia. *Assistant Professor of Physiology.* (1, 1925)

McCrea, Forrest D., Ph.D. Duke University School of Medicine, Durham, N. C. *Associate Professor of Physiology and Pharmacology.* (1, 1929; 3, 1937)

McCrudden, F. H., M.D. 501 Boylston St., Boston, Mass. *Assistant Medical Director, New England Mutual Life Insurance Co.* (2, 1906)

McCullagh, D. Roy, Ph.D. 2040 E. 93rd St., Cleveland, O. *Director, Department of Biochemistry, Cleveland Clinic Foundation.* (2, 1932)

McCulloch, Warren Sturgis, M.A., M.D. University of Illinois, College of Medicine, Chicago. *Associate Professor of Psychiatry.* (1, 1936)

McCutcheon, Morton, M.D. University of Pennsylvania Medical School, Philadelphia. *Professor of Pathology.* (4, 1925)

McDonald, Claude H., D.Sc. 5707 T St., Little Rock, Ark. *Head, Department of Physiology and Pharmacology, Arkansas University School of Medicine.* (1, 1936)

McDonald, Francis Guy, M.S., Ph.D. Research Laboratory, Mead Johnson & Co., Evansville, Ind. *Research Biochemist.* (2, 1936)

McEllroy, William Swindler, M.D. School of Medicine, University of Pittsburgh, Pittsburgh, Pa. *Professor of Physiological Chemistry; Dean, School of Medicine.* (2, 1919)

McFarlane, William Douglas, Ph.D. Macdonald College, (McGill University), Macdonald College, P. Q., Canada. *Professor of Chemistry.* (2, 1933)

McGinty, Daniel A., M.A., Ph.D. Parke, Davis & Co., Detroit, Mich. *Research Physiologist.* (1, 1925)

McGuigan, Hugh Alister, Ph.D., M.D. 1853 W. Polk St., Chicago, Ill. *Professor of Pharmacology and Therapeutics, College of Medicine, University of Illinois.* (1, 1907; 2, 1906; 3, 1913)

McHargue, J. S., M.S., Ph.D., D.Sc. 411 Transylvania Park, Lexington, Ky. *Head, Department of Chemistry, Kentucky Agricultural Experiment Station.* (2, 1927)

McHenry, E. W., M.A., Ph.D., F.R.S.C. School of Hygiene, University of Toronto, Toronto, Canada. *Assistant Director, Connaught Laboratories; Associate Professor in Charge of Nutrition.* (2, 1938; 5, 1935)

McIntyre, A. R., Ph.D., M.D. College of Medicine, University of Nebraska, 42nd and Dewey Ave., Omaha. *Professor of Physiology and Pharmacology.* (1, 1933; 3, 1938)

McKee, Clara M., Squibb Institute for Medical Research, New Brunswick, N. J. *Assistant in Microbiology.* (6, 1941)

McLain, Paul L., M.D. University of Pittsburgh Medical School, Pittsburgh, Pa. *Assistant*

Professor of Physiology and Pharmacology. (3, 1940)

McLean, Franklin C., Ph.D., M.D. University of Chicago, Chicago, Ill. *Professor of Pathological Physiology.* (1, 1914; 2, 1916; 3, 1916)

McLester, James S., M.D., LL.D. University of Alabama, 930 S. 20th St., Birmingham. *Professor of Medicine.* (5, 1933)

McMaster, Philip D., M.D. The Rockefeller Institute for Medical Research, 66th St. and York Ave., New York City. (4, 1924)

McMeekin, Thomas L., Ph.D. Eastern Regional Research Laboratory, U. S. Department of Agriculture, Philadelphia, Pa. *Senior Chemist.* (2, 1935)

MacNabb, Andrew L., V.S., B.V.Sc., F.A.P.H.A. Department of Health of Ontario, Toronto, Canada. *Director of Laboratories.* (6, 1941)

McNaught, James B., A.M., M.D. Stanford University School of Medicine, San Francisco, Calif. *Associate Professor of Pathology.* (4, 1936)

McPhail, Murchie Kilburn, Ph.D. Dalhousie University, Halifax, Nova Scotia. *Professor of Pharmacology.* (3, 1941)

McQuarrie, Irvine, Ph.D., M.D. University of Minnesota, Minneapolis. *Professor and Head of Department of Pediatrics.* (4, 1927; 5, 1933)

Medes, Grace, Ph.D. Lankenau Hospital Research Institute, Philadelphia, Pa. *Research Physiological Chemist.* (2, 1930)

Medlar, Edgar M., M.D. Metropolitan Life Insurance Co. Sanatorium, Mt. McGregor, N. Y. *Pathologist.* (4, 1927)

Meek, Walter J., Ph.D. University of Wisconsin, Madison. *Professor of Physiology; Assistant Dean of the Medical School.* (1, 1908)

Mellon, Ralph R., M.D., M.Sc., Dr. P.H., Sc.D. (hon.). Institute of Pathology, Western Pennsylvania Hospital, Pittsburgh. *Director.* (6, 1918)

Melnick, Daniel, Ph.D. Food Research Laboratories, Inc., 48–14 33rd St., Long Island City, N. Y. *Chief Chemist.* (2, 1940; 5, 1942)

Melville, Kenneth Ivan, M.Sc., M.D., C.M. McGill University, Montreal, Canada. *Assistant Professor of Pharmacology.* (3, 1931)

Mendenhall, Walter L., S.M., M.D. Boston University Medical School, 80 E. Concord St., Boston, Mass. *Professor of Pharmacology.* (1, 1915; 3, 1917)

Menkin, Valy, M.A., M.D. Harvard Medical School, 25 Shattuck St., Boston, Mass. *Assistant Professor of Pathology.* (1, 1932; 4, 1932; 6, 1931)

Menten, Maud L., M.D., Ph.D. University of Pittsburgh, Pittsburgh, Pa. *Associate Professor of Pathology.* (1, 1915; 4, 1927)

Menzel, Arthur E. O., Ph.D. The Squibb Institute for Medical Research, New Brunswick, N. J. *Associate.* (6, 1939)

Mettier, Stacy R., M.D. University of California Hospital, San Francisco. *Associate Professor of Medicine.* (4, 1932)

Mettler, Fred A., A.M., Ph.D., M.D. College of Physicians and Surgeons, Columbia University, New York City. *Associate Professor of Anatomy.* (1, 1937)

Meyer, Curtis E., M.S., Ph.D. The Upjohn Co., Kalamazoo, Mich. *Research Chemist.* (2, 1942)

Meyer, Karl, M.D., Ph.D. 630 W. 168th St., New York City. *Associate Professor of Biological Chemistry, College of Physicians and Surgeons, Columbia University.* (2, 1934)

Meyer, Karl F., M.D., Ph.D. Medical Center, San Francisco, Calif. *Professor of Bacteriology, University of California. Director of the George Williams Hooper Foundation for Medical Research.* (4, 1930; 6, 1922)

Meyerhof, Otto, M.D., LL.D. Department of Physiological Chemistry, University of Pennsylvania School of Medicine, Philadelphia. *Research Professor of Biochemistry.* (2, 1941)

Michaelis, Leonor, M.D. Rockefeller Institute for Medical Research, 66th St. and York Ave., New York City. *Member Emeritus.* (2, 1929)

Mider, George Burroughs, M.D. Cornell University Medical College, 1300 York Ave., New York City. *Assistant Professor of Pathology.* (4, 1940)

Miles, Walter R., A.M., Ph.D. 333 Cedar St., New Haven, Conn. *Professor of Psychology, The School of Medicine and the Institute of Human Relations, Yale University; Member of the National Academy of Sciences.* (1, 1919)

Milhorat, Ade T., M.D. Cornell University Medical College, 1300 York Ave., New York City. *Assistant Professor of Medicine and Instructor in Pharmacology; Research Fellow, Russell Sage Institute of Pathology.* (1, 1934; 3, 1937; 5, 1935)

Miller, B. F., Ch.E., M.D. University of Chicago, Chicago, Ill. *Assistant Professor of Medicine; Research Associate, Walter G. Zoller Dental Clinic.* (2, 1938)

Miller, Carey D., University of Hawaii, Honolulu. *Professor of Food and Nutrition, Hawaii Agricultural Experimental Station.* (5, 1942)

Miller, C. Phillip, M.D., M.S. University of Chicago, Chicago, Ill. *Professor of Medicine.* (4, 1925; 6, 1928)

Miller, Edgar C. L., M.D. %Library, Medical College of Virginia, Richmond. *Directing Librarian.* (6, 1913)

Miller, Edgar G., Jr., Ph.D. 630 W. 168th St., New York City. *Professor of Biological Chemistry, Columbia University.* (2, 1930)

Miller, Franklin R., M.D. Jefferson Medical College and Hospital, Division of Hematology, Philadelphia, Pa. *Associate Professor of Medicine.* (4, 1940)

Miller, Frederick R., A.M., M.D., F.R.C.P. (C), F.R.S. Faculty of Medicine, University of Western Ontario, London, Ont., Canada. *Professor of Physiology.* (1, 1908)

Miller, G. H., A.M., M.D. American University of Beirut, Beirut, Syria. *Dean of the College of Medicine.* (3, 1925)

Miller, Lloyd C., Ph.D. Division of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D. C. *Senior Pharmacologist.* (3, 1938)

Miller, R. C., Ph.D. Pennsylvania State College, State College. *Assistant Professor Agricultural and Biological Chemistry.* (5, 1935)

Miller, Zelma Baker, Ph.D. Department of Medicine, University of Chicago, Chicago, Ill. *Research Instructor.* (2, 1940)

Millikan, Glenn A., Ph.D. Johnson Foundation, University of Pennsylvania, Philadelphia. *Fellow in Biophysics.* (1, 1940)

Mills, Clarence A., Ph.D., M.D. 5046 Oberlin Blvd., Cincinnati, O. *James T. Heady Professor of Experimental Medicine, University of Cincinnati.* (1, 1921; 2, 1921)

Minot, Annie Stone, Ph.D. Vanderbilt University Medical School, Nashville, Tenn. *Research Associate, Department of Pharmacology.* (1, 1923)

Mirsky, Alfred E., Ph.D. Hospital of the Rockefeller Institute, 66th St. and York Ave., New York City. *Associate Member, Rockefeller Institute.* (2, 1941)

Mirsky, I. Arthur, M.Sc., M.D., C.M. The Jewish Hospital, Cincinnati, O. *Director, The May Institute for Medical Research; Assistant Professor of Biochemistry, University of Cincinnati.* (1, 1936)

Mitchell, Harold H., M.S., Ph.D. Room 557, Old Agricultural Bldg., University of Illinois, Urbana. *Professor of Animal Nutrition.* (2, 1919; 5, 1933)

Mitchell, Helen S., Ph.D. Nutrition Division, Office of Defense Health and Welfare Service, Washington, D. C. *Principal Nutritionist.* (2, 1925; 5, 1933)

Mitchell, Philip H., Ph.D. Brown University, Providence, R. I. *Professor of Physiology.* (2, 1909)

Molitor, Hans, M.D. 50 Lawrence St., Rahway, N. J. *Director, Merck Institute for Therapeutic Research.* (1, 1933; 3, 1942)

Molomut, Norman, M.A., Ph.D. %U. S. Army, Wright Field, Dayton, O. *Assistant Bacteriologist, Department of Medicine, Columbia University (on leave); First Lieutenant, Army of U. S. Aero Medical Research.* (6, 1942)

Moon, Virgil H., M.Sc., M.D. Jefferson Medical College, Philadelphia, Pa. *Professor of Pathology.* (4, 1934)

Moore, A. R., Ph.D. University of Oregon, Eugene. *Research Professor of General Physiology in the Department of Psychology.* (1, 1912)

Moore, Carl Vernon, M.D. Washington University School of Medicine, St. Louis, Mo. *Associate Professor of Medicine.* (4, 1938; 5, 1941)

Moore, Lane A., Ph.D. University of Maryland, College Park. *Research Assistant in Dairy Husbandry.* (5, 1940)

Moore, Robert A., M.D. Washington University Medical School, St. Louis, Mo. *Professor of Pathology.* (4, 1929)

Moore, Robert M., M.D. Station Hospital, Camp Bowie, Texas. *Lt. Col., M.C.; Associate Professor of Surgery, University of Texas Medical School, Galveston.* (1, 1932)

Moorhouse, Victor Henry K., M.B. University of Manitoba, Winnipeg, Canada. *Professor of Physiology.* (1, 1912)

Morgan, Agnes Fay, M.S., Ph.D. University of California, Berkeley. *Professor of Home Economics; Biochemist, Agric. Exp. Station; Head, Department of Home Economics.* (2, 1929; 5, 1933)

Morgulis, Sergius, A.M., Ph.D. University of Nebraska College of Medicine, Omaha. *Professor of Biochemistry.* (1, 1914; 2, 1916)

Morison, Robert S., M.D. Harvard Medical School, Boston, Mass. *Assistant Professor of Anatomy.* (1, 1938)

Moritz, Alan R., M.D. Harvard Medical School, Boston, Mass. *Professor of Legal Medicine.* (4, 1934)

Morrell, Clarence Allison, M.A., Ph.D. Department of Pensions and National Health, Laboratory of Hygiene, Sussex and John Sts., Ottawa, Canada. *Senior Pharmacologist.* (3, 1937)

Morris, Marion C., M.S., Ph.D. Medical School, Washington University, Scott Ave., St. Louis, Mo. *Assistant Professor of Bacteriology and Immunology.* (6, 1936)

Morrison, Dempsie B., M.S., Ph.D. University of Tennessee College of Medicine, Memphis. *Associate Professor of Chemistry.* (2, 1936)

Morse, Minerva, M.S., Ph.D. 5525 Kimbark Ave., Chicago, Ill. *Research Associate, Department of Pediatrics, University of Chicago.* (2, 1934)

Morse, Withrow, Ph.D. 32 Manchester Rd., Eastchester, via Tuckahoe, N. Y. *Consultant.* (2, 1914)

Mortimer, Bernard, Ph.D., M.D. Cook County Hospital, Chicago, Ill. (1, 1936)

Morton, John J., M.D. University of Rochester, School of Medicine and Dentistry, Rochester, N. Y. *Professor of Surgery.* (4, 1927)

Mosenthal, Herman O., M.D. 889 Lexington Ave., New York City. *Professor of Medicine, New York Post-Graduate Medical School.* (2, 1915)

Moulton, C. Robert, Ph.D. 5717 Kenwood Ave., Chicago, Ill. *Editor.* (5, 1933)

Mudd, Stuart, M.A., M.D. University of Pennsylvania, Philadelphia. *Professor of Bacteriology.* (1, 1921; 4, 1927; 6, 1927)

Muehlberger, Clarence W., M.S., Ph.D. State Health Department Laboratories, Lansing, Mich. *State Toxicologist.* (3, 1928)

Mueller, J. Howard, M.S., Ph.D. 2176 Centre St., W. Roxbury, Mass. *Professor of Bacteriology and Immunology, Harvard Medical School.* (2, 1922; 4, 1927; 6, 1920)

Mukherji, B., M.B., D.Sc. All-India Institute of Hygiene and Public Health, Calcutta. *Director, Biochemical Standardization Laboratryo.* (3, 1938)

Mulder, Arthur G., Ph.D. University of Tennessee College of Medicine, Memphis. *Associate Professor of Physiology.* (1, 1937)

Mulinos, M. G., M.D., Ph.D. College of Physicians and Surgeons, Columbia University, 630 W. 168th St., New York City. *Associate Professor of Pharmacology.* (3, 1931)

Mull, James W., Ph.D. Maternity Hospital, 2065 Adelbert Rd., Cleveland, O. *Senior Instructor in Biochemistry in charge of Biochemical Research in Obstetrics, Western Reserve University.* (2, 1937)

Mullin, F. J., M.S., Ph.D. University of Chicago, Chicago, Ill. *Instructor in Physiology.* (1, 1937)

Munsell, Hazel E., M.A., Ph.D. Havemeyer Hall, Columbia University, New York City. (5, 1933)

Muntwyler, Edward, Ph.D. Western Reserve University, 2109 Adelbert Rd., Cleveland, O. *Associate Professor of Biochemistry.* (2, 1931)

Murlin, John R., A.M., Ph.D., Sc.D. University of Rochester Medical School, 260 Crittenden Blvd., Rochester, N. Y. *Lewis P. Ross Professor of Physiology and Director of Department of Vital Economics.* (1, 1906; 2, 1908; 5, 1933)

Murphy, James B., M.D. Rockefeller Institute for Medical Research, 66th St. and York Ave., New York City. *Member.* (4, prior to 1920)

Murray, Everitt G. D., O.B.E., B.A. honors in Natural Science, M.A., L.M.S.S.A., F.R.S.C. McGill University, Montreal, Canada. *Professor of Bacteriology and Immunology and Head of the Department, McGill University; Bacteriologist-in-Chief to the Royal Victoria Hospital, to the Children's Memorial Hospital and to the Alexandra Hospital.* (6, 1933)

Myers, Chester N., Ph.D., Sc.D. 170 Varick St., New York City. *Chief, Division Chemotherapy, N. Y. Skin and Cancer Hospital; Associate in Dermatology and Syphilology, College of Physicians and Surgeons; Research Chemist, Vanderbilt Clinic; Director, Chemical and Clinical Research, H. A. Metz Laboratories, Inc.* (2, 1922)

Myers, Victor C., M.A., Ph.D., Sc.D. School of Medicine, Western Reserve University, Cleveland, O. *Professor and Director of Biochemistry.* (1, 1916; 2, 1910; 5, 1933)

Nachmansohn, David, M.D. Laboratory of Physiology, School of Medicine, Yale University, New Haven, Conn. (1, 1940)

Nadler, J. Ernest, M.D., Med.D.Sc. 477 First Ave., New York City. *Instructor in Medicine.* (3, 1940)

Nahum, Louis N., M.D. 1142 Chapel St., New Haven, Conn. *Assistant Professor of Physiology, Yale University.* (1, 1934)

Nash, Thomas P., Jr., M.A., Ph.D. 875 Monroe Ave., Memphis, Tenn. *Professor of Chemistry, College of Medicine; Dean of School of Biological Sciences, University of Tennessee.* (2, 1923)

Nasset, Edmund S., M.S., Ph.D. University of Rochester, 260 Crittenden Blvd., Rochester, N. Y. *Associate Professor of Physiology; Major, San. Corps., Miami Beach, Fla.* (1, 1932; 5, 1940)

Nathanson, Morris D., M.D. 658 S. Bonnie Brae St., Los Angeles, Calif. *Associate Clinical Professor of Medicine, University of Southern California School of Medicine.* (3, 1940)

Necheles, Heinrich, M.D., Ph.D. Michael Reese Hospital, Chicago, Ill. *Director, Dept. of Gastro-intestinal Physiology, Michael Reese Hospital; Professorial Lecturer in Physiology, University of Chicago.* (1, 1929)

Neill, James M., Ph.D. Medical College, Cornell University, 1300 York Ave., New York City. *Professor of Bacteriology and Immunology.* (6, 1930)

Neilson, Charles Hugh, A.M., Ph.D., M.D. Humboldt Building, St. Louis, Mo. *Associate Dean and Professor of Medicine, St. Louis University Medical School.* (2, 1906)

Nelson, Arthur A., M.D., Ph.D. Food and Drug Administration, Federal Security Agency, Washington, D. C. *Senior Pathologist, Division of Pharmacology.* (4, 1942)

Nelson, Carl Ferdinand, M.D., Ph.D. Department of Biochemistry, University of Kansas, Lawrence. *Professor of Physiological Chemistry.* (2, 1914)

Nelson, Erwin E., Ph.D., M.D. Tulane University, Station 20, New Orleans, La. *Professor of Pharmacology.* (1, 1923; 3, 1924)

Nelson, E. M., M.S., Ph.D. Food and Drug Administration, Federal Security Agency, Washington, D. C. *Chief, Vitamin Division.* (2, 1927)

Nelson, John B., Ph.D. Rockefeller Institute for Medical Research, Princeton, N. J. *Associate Member.* (4, 1934)

Nelson, John M., Ph.D. Columbia University, New York City. *Professor of Organic Chemistry.* (2, 1923)

Nelson, P. Mabel, M.S., Ph.D. Iowa State College, Ames. *Professor and Head of Department of Foods and Nutrition.* (5, 1934)

Nelson, Tell, M.A., M.D. 1st Station Hospital, A.P.O. 915, % Postmaster, San Francisco, Calif. *Major, M. C., U. S. A.* (6, 1938)

Nelson, Victor E., M.S. Iowa State College, Ames. *Professor of Physiological Chemistry.* (2, 1924; 5, 1933);

Nelson, Warren O., M.S., Ph.D. Wayne University College of Medicine, Detroit, Mich. *Professor of Anatomy.* (1, 1937)

Neter, Erwin, M.D. School of Medicine, University of Buffalo, 24 High St., Buffalo, N. Y. *Attending Bacteriologist, Children's Hospital.* (6, 1937)

Nettleship, Anderson, M.D. National Cancer Institute, National Institute of Health, U. S. Public Health Service, Bethesda, Md. *Passed Assistant Surgeon.* (R) (4, 1942)

Neurath, Hans, Ph.D. School of Medicine, Duke University, Durham, N. C. *Assistant Professor of Biochemistry.* (2, 1940)

Neuwelt, Frank, M.D. 504 Broadway, Gary, Ind. *Research Associate, Department of Gastrointestinal Research, Michael Reese Hospital.* (1, 1940)

Neuwirth, Isaac, Ph.D. 209 E. 23rd St., New York City. *Associate Professor of Pharmacology and Therapeutics, New York University College of Dentistry.* (2, 1924; 3, 1931)

Newburgh, L. H., M.D. University of Michigan, Ann Arbor. *Professor Clinical Investigation, Medical School.* (5, 1933)

Nice, Leonard B., Ph.D. Chicago Medical School, 710 S. Wolcott Ave., Chicago, Ill. *Professor of Physiology and Pharmacology.* (1, 1921)

Nicholas, John S., M.S., Ph.D. Osborn Zoological Laboratory, Yale University, New Haven, Conn. *Bronson Professor of Comparative Anatomy.* (1, 1927)

Nicholson, Hayden C., M.S., M.D. University of Michigan, Ann Arbor. *Associate Professor of Physiology.* (1, 1932)

Nicolet, Ben H., Ph.D. Bureau of Dairy Industry, U. S. Department of Agriculture, Beltsville, Md. *Senior Chemist.* (2, 1932)

Niemann, Carl G., Ph.D. Crellin-Gates Chemical Laboratory, Pasadena, Calif. *Assistant Professor Organic Chemistry, California Institute of Technology.* (2, 1940)

Nigg, Clara, M.A., Ph.D. % E. R. Squibb & Sons, New Brunswick, N. J. *In charge of Virus Division, Biological Laboratories.* (6, 1929)

Nims, Leslie F., M.A., Ph.D. Yale University School of Medicine, 333 Cedar St., New Haven, Conn. *Assistant Professor of Physiology.* (1, 1940)

Noble, Robert Laing, M.D., Ph.D. Research Institute of Endocrinology, McGill University, Montreal, Canada. *Research Assistant.* (1, 1941)

Nord, F. F., Ph.D. Fordham University, New York City. *Professor of Chemistry.* (2, 1940)

Norris, Earl R., Ph.D. University of Washington, Seattle. *Professor of Chemistry.* (2, 1938)

Norris, L. C., Ph.D. Rice Hall, Cornell University, Ithaca, N. Y. *Professor of Nutrition; Secretary, School of Nutrition.* (2, 1939; 5, 1934)

Northrop, J. H., M.A., Ph.D., Sc.D., LL.D. Rockefeller Institute for Medical Research, Princeton, N. J. *Member.* (2, 1938)

Northup, David W., M.A., Ph.D. West Virginia University Medical School, Morgantown. *Associate Professor of Physiology.* (1, 1936)

Novy, F. G., M.D., Sc.D., LL.D. 721 Forest Ave., Ann Arbor, Mich. *Dean Emeritus of the Medical School and Professor Emeritus of Bacteriology, University of Michigan; Member, National Academy of Sciences.* (2, 1906)

Nye, Robert N., M.D. 32 Lawrence Rd., Chestnut Hill, Mass. *Editor, New England Journal of Medicine.* (6, 1923)

Oberst, Fred W., M.S., Ph.D. U. S. Public Health Service Hospital, Lexington, Ky. *Biological Chemist.* (2, 1936)

Ochoa, Severo, M.D. New York University College of Medicine, New York City. *Research Associate in Biochemistry.* (2, 1942)

Ogden, Eric, M.R.C.S. (England), L.R.C.P. (London). University of California, Berkeley. *Assistant Professor of Physiology.* (1, 1941)

O'Hare, James P., M.D. 520 Commonwealth Ave., Boston, Mass. *Physician, Peter Bent Brigham Hospital; Assistant Professor of Medicine, Harvard Medical School.* (4, 1927)

Okey, Ruth, Ph.D. 1583 Life Sciences Bldg., University of California, Berkeley. *Associate Professor of Home Economics; Associate Biochemist, State Exp. Station, College of Agriculture.* (2, 1922; 5, 1933)

Olcott, Harold S., M.S., Ph.D. Western Regional Research Laboratory, U. S. Department of Agriculture, Albany, Calif. *Senior Chemist.* (2, 1935)

Oldham, Frances Kathleen, M.S., Ph.D. University of Chicago, Chicago, Ill. *Research Assistant in Pharmacology.* (3, 1941)

Olitsky, Peter K., M.D. Rockefeller Institute for Medical Research, 66th St. and York Ave., New York City. *Member.* (4, 1923; 6, 1917)

Oliver, Jean Redman, M.D. Hoagland Laboratory, 335 Henry St., Brooklyn, N. Y. *Professor of Pathology, Long Island College of Medicine.* (1, 1924; 4, 1924)

Oliver, Wade W., M.D. Hoagland Laboratory, 335 Henry St., Brooklyn, N. Y. *Professor of Bacteriology, Long Island College of Medicine.* (4, 1925)

Olmsted, J. M. D., M.A., Ph.D. University of California, Berkeley. *Professor of Physiology.* (1, 1920)

Olson, Carl, Jr., D.V.M., Ph.D. Massachusetts State College, Amherst. *Research Professor of Veterinary Science.* (4, 1937)

Opie, Eugene L., M.D., Sc.D., LL.D. Rockefeller Institute for Medical Research, 66th St. and York Ave., New York City. *Professor Emeritus of Pathology, Cornell University Medical College. Member, National Academy of Sciences.* (1, 1906; 4, prior to 1920; 6, 1923)

Oppenheimer, Enid Tribe. 124 E. 61st St., New York City. *Instructor in Physiology, Columbia University.* (1, 1932)

Oppenheimer, Morton Joseph, Ed.M., M.D. 3400 N. Broad St., Philadelphia, Pa. *Associate Professor of Physiology, Temple University School of Medicine.* (1, 1942)

Orent-Keiles, Elsa, D.Sc. Bureau of Home Economics, U. S. Department of Agriculture, Beltsville, Md. *In Charge of Nutrition Investigations; Assistant Chief, Foods and Nutrition Division.* (2, 1935; 5, 1935)

Ort, John M., Ph.D. 356 Raymond St., Rockville Centre, Long Island, N. Y. *Research Chemist, E. R. Squibb and Sons Co.* (2, 1932)

Orten, James M., M.S., Ph.D. Wayne University College of Medicine, Detroit, Mich. *Assistant Professor of Physiological Chemistry.* (2, 1936; 5, 1937)

Orth, O. Sidney, M.S., Ph.D., M.D. University of Wisconsin Medical School, Madison. *Instructor in Physiology.* (1, 1942)

Osborne, Stafford L., B.P.E., M.S., Ph.D. Northwestern University Medical School, 303 E. Chicago Ave., Chicago, Ill. *Assistant Professor of Physical Therapy.* (1, 1941)

Oster, Robert H., Ph.D. University of Maryland Medical School, Greene and Lombard Sts., Baltimore. *Assistant Professor of Physiology.* (1, 1938)

Osterberg, Arnold E., M.S., Ph.D. Mayo Clinic, Rochester, Minn. *Head, Clinical Biochemistry; Associate Professor, Mayo Foundation.* (2, 1933)

Osterhout, W. J. V., Ph.D. Rockefeller Institute, 66th St. and York Ave., New York City. *Member Emeritus of the Institute; Member of the National Academy of Sciences.* (1, 1910)

Owen, Seward E., M.S., Ph.D. Headquarters 4th Corps Area, Atlanta, Ga. *Capt., San. Corps; Physiologist, Cancer Research Unit, Veteran's Administration, Hines, Ill. (on leave).* (1, 1938)

Pack, George T., M.D. 155 East 72nd St., New York City. *Fellow in Cancer Research, Memorial Hospital.* (1, 1924)

Page, Irvine H., M.D. Indianapolis City Hospital, Indianapolis, Ind. *Director of Clinical Research.* (1, 1937; 2, 1932)

Painter, Elizabeth E., Ph.D. College of Physicians and Surgeons, Columbia University, 630 W. 168th St., New York City. *Instructor in Physiology.* (1, 1941)

Palmer, Albert H., Ph.D. Pennsylvania State College School of Agriculture, State College. *Assistant Professor of Biochemistry.* (2, 1934)

Palmer, Leroy S., A.M., Ph.D. Biochemical Building, University Farm, St. Paul, Minn *Professor of Agricultural Biochemistry, University of Minnesota; Dairy Chemist, Minnesota Agricultural Experiment Station.* (2, 1920; 5, 1933)

P'An, S. Y., M.D. Peiping Union Medical College, Peiping, China. *Assistant in Pharmacology.* (3, 1941)

Pangborn, Mary C., Ph.D. 20 Morris St., Albany, N. Y. *Assistant Biochemist, New York State Department of Health, Division of Laboratories and Research.* (2, 1941)

Pappenheimer, Alwin M., Jr., Ph.D. New York University, College of Medicine, New York City. *Assistant Professor of Bacteriology.* (2, 1941)

Pappenheimer, Alwin M., M.D. 630 W. 168th St., New York City. *Professor of Pathology, Columbia University.* (4, 1922; 6, 1938)

Park, Edwards A., M.D. Johns Hopkins Hospital, Baltimore, Md. *Professor of Pediatrics, Johns Hopkins University.* (4, 1923)

Parker, George Howard, Sc.D. 16 Berkeley St., Cambridge, Mass. *Professor of Zoology Emeritus, Harvard University; Member of the National Academy of Sciences.* (1, 1900)

Parker, Robert F., M.D. Lakeside Hospital, 2065 Adelbert Rd., Cleveland, O. *Associate Professor of Medicine.* (4, 1942; 6, 1935)

Parkins, William M., M.A., Ph.D. School of Medicine, University of Pennsylvania, Philadelphia. *Research Fellow, Harrison Department of Surgical Research.* (1, 1939)

Parpart, Arthur K., Ph.D. Guyot Hall, Princeton University, Princeton, N. J. *Associate Professor of Physiology.* (1, 1937)

Parr, Leland W., Ph.D. The George Washington University School of Medicine, 1335 H St., N.W. Washington, D. C. *Professor of Bacteriology.* (4, 1940)

Parsons, Helen T., M.S., Ph.D. University of Wisconsin, Madison. *Professor of Home Economics; In Charge of Purnell Research in Nutrition.* (2, 1929; 5, 1933)

Parsons, Robert J., M.D. University of Michigan, Ann Arbor. *Assistant Professor of Pathology.* (4, 1939)

Paschkis, Karl E., M.D. 1025 Walnut St., Philadelphia, Pa. *J. Ewing Mears Fellow in Physiology and Medicine, Jefferson Medical College; Chief Clinical Assistant, Endocrine Clinic, Jefferson Medical College Hospital.* (1, 1942)

Patterson, Thos. L., A.M., M.S., Ph.D. Wayne University College of Medicine, 1512 St. Antoine St., Detroit, Mich. *Professor of Physiology.* (1, 1920)

Paul, John R., M.D., A.M. 330 Cedar St., New Haven, Conn. *Professor of Preventive Medicine, Yale University Medical School.* (4, 1927; 6, 1937)

Pearce, John Musser, M.D. Long Island College of Medicine, Hoagland Laboratory, 335 Henry St., Brooklyn, N. Y. *Assistant Professor of Pathology.* (4, 1942)

Pearce, Louise, M.D. Rockefeller Institute for Medical Research, Princeton, N. J. *Associate Member in Pathology and Bacteriology.* (3, 1915; 4, 1925)

Pearcy, Frank, Ph.D., M.D. 471 Park Ave., New York City. (1, 1928)

Pearse, Herman E., M.D. School of Medicine and Dentistry, University of Rochester, Crittenden Blvd., Rochester, N. Y. *Associate Professor of Surgery.* (4, 1932)

Pearson, Paul B., Ph.D. Texas A. & M. College, College Station. *Professor in charge of Animal Nutrition.* (5, 1940)

Pease, Marshall C., Jr., M.D. 155 E. 62nd St., New York City. *Clinical Professor of Pediatrics, New York Post-Graduate Medical School and Hospital, Columbia University.* (6, 1920)

Pemberton, Ralph, M.S., M.D. University of Pennsylvania, Philadelphia. *Professor of Medicine, Graduate School of Medicine.* (5, 1933)

Penfield, Wilder G., M.D., D.Sc. McGill University, Montreal, Que., Canada. *Professor of Neurology and Neurosurgery.* (1, 1932)

Pennington, Mary Engle, Ph.D. 233 Broadway, New York City. *Consultant in Connection with the Handling, Transportation and Storage of Perishables.* (2, 1908)

Peoples, S. Anderson, M.D. University of Alabama School of Medicine, University. *Associate Professor of Physiology and Pharmacology.* (3, 1937)

Perlzweig, William A., A.M., Ph.D. Box 3711, Duke Hospital, Durham, N. C. *Professor of Biochemistry, Duke University; Biochemist, Duke Hospital.* (2, 1924)

Permar, Howard H., M.D. Pathologic Laboratories, Mercy Hospital, Pittsburgh, Pa. *Director of Laboratories.* (4, 1925)

Peters, John P., M.A., M.D. Yale University School of Medicine, New Haven, Conn. *John Slade Ely Professor of Medicine.* (2, 1922)

Petersen, William F., M.D. 1322 Astor St., Chicago, Ill. *Professor of Pathology, University of Illinois.* (3, 1923; 4, 1923)

Peterson, William H., A.M., Ph.D. Biochemistry Building, University of Wisconsin, Madison. *Professor of Biochemistry.* (2, 1919; 5, 1936)

Petroff, S. A., Ph.D., Sc.D. Sea View Hospital, West New Brighton, Staten Island, N. Y. *Director of Bacteriology and Immunology.* (6, 1926)

Pett, L. B., M.D., Ph.D. Nutrition Services, Department of Pensions and National Health, Ottawa, Canada. *Director.* (2, 1937)

Peugnet, Hubert B., M.D. School of Medicine, Washington University, Euclid Ave. and Kingshighway, St. Louis, Mo. *Assistant Professor in Physiology.* (1, 1938)

Pfeiffer, Carl C., Ph.D., M.D. Parke, Davis & Co., Detroit, Mich. *Chief Pharmacologist. Lecturer in Pharmacology, Wayne University School of Medicine.* (3, 1938)

Pfiffner, Joseph J., Ph.D. 1007 Lincoln Ave., Ann Arbor, Mich. *Research Chemist, Parke, Davis & Co., Detroit.* (1, 1931; 2, 1931)

Phatak, Nilkanth M., M.S., Ph.D. North Pacific College of Oregon, School of Dentistry, Portland. *Associate Professor of Physiology, Pharmacology, and Research; and Instructor, Dept. of Pharmacology, University of Oregon Medical School, Portland.* (3, 1941)

Phillips, Paul H., Ph.D. University of Wisconsin, Madison. *Professor of Biochemistry.* (2, 1940; 5, 1938)

Phillips, Robert Allan, M.D. Rockefeller Institute for Medical Research, New York City. *Fellow.* (1, 1938)

Pick, Ernst Peter, M.D. 19 E. 98th St., New York City. *Associate Pharmacologist to the Mt. Sinai Hospital; Clinical Professor of Pharmacology in Columbia University.* (3, 1940)

Pierce, Harold B., M.S., Ph.D. College of Medicine, University of Vermont, Burlington. *Professor and Head of Physiological Chemistry.* (2, 1929; 5, 1933)

Pierce, Harold Fisher, Ph.D., M.D. 65 E. 55th St., New York City. *Instructor in Post-Graduate Medicine, Columbia University; Major, San. Corp., Randolph Field, Texas.* (1, 1928)

Pierce, Ira H., M.S., Ph.D. Univ. of Iowa, Iowa City. *Associate Professor of Pharmacology.* (3, 1933)

Pike, Frank H., Ph.D. 630 W. 168th St., New York City. *Associate Professor of Physiology, Columbia University.* (1, 1907)

Pilcher, J. Douglas, M.D. City Hospital, Scranton Road, Cleveland, O. *Associate Professor of Pediatrics, Western Reserve Medical School.* (1, 1912; 3, 1911)

Pillemer, Louis, Ph.D. Box 195, Room 111, Army Medical School, Army Medical Center, Washington, D. C. *First Lieutenant, Sanitary Corps, U. S. A.* (6, 1942)

Pincus, Gregory, M.S., Sc.D. Clark University, Worcester, Mass. *Visiting Professor of Experimental Zoology.* (1, 1935)

Pinkerton, Henry, M.D. St. Louis University School of Medicine, St. Louis, Mo. *Professor of Pathology.* (4, 1931)

Pinkston, James O., Ph.D. Division of Industrial Hygiene, National Institute of Health, Bethesda, Md. *Pharmacologist.* (1, 1936; 3, 1939)

Pittman, Martha S., A.M., Ph.D. Kansas State College, Manhattan. *Head of Department of Food Economics and Nutrition.* (5, 1933)

Pitts, Robert F., Ph.D., M.D. New York University College of Medicine, 477 First Ave., New York City. *Assistant Professor of Physiology.* (1, 1934)

Plass, Everett D., M.D. University Hospital, Iowa City, Iowa. *Professor and Head of Department of Obstetrics and Gynecology, State University of Iowa.* (2, 1922)

Plotz, Harry, M.D. Army Medical School, Army Medical Center, Washington, D. C. *Chief of Virus and Rickettsial Laboratory.* (6, 1917)

Pohlman, Augustus G., M.D. 2202 W. Third St., Los Angeles, Calif. *Associate Clinical Professor, Department of Otolaryngology, University of Southern California School of Medicine.* (1, 1934)

Pollack, Herbert, Ph.D., M.D. 20 E. 76th St., New York City. *Associate in Medicine and Physician in Charge of Metabolism Clinics, Mt. Sinai Hospital.* (1, 1933; 5, 1935)

Pond, Samuel E., A.M., Ph.D. U. S. Naval Research Laboratory, Anacostia Station, Washington, D. C. 1203 Enfield St., R. F. D., Thompsonville, Conn. (1, 1924)

Ponder, Eric, M.D., Sc.D. The Nassau Hospital, Mineola, Long Island, N. Y. (1, 1931)

Popper, Hans, M.S., M.D. University of Illinois College of Medicine, 1825 W. Harrison St., Chicago. *Instructor in Pathology; Director of Clinical Laboratories of Cook County Hospital.* (4, 1942)

Porter, Eugene L., A.M., Ph.D. University of Texas, Medical Branch, Galveston. *Professor of Physiology.* (1, 1913)

Porter, William Townsend, M.D., Sc.D., LL.D. Dover, Mass. *Professor Emeritus of Comparative Physiology, Harvard University.* (1, 1891)

Potter, Truman S., M.D. University of Chicago, Chicago, Ill. *Research Associate in Preventive Medicine.* (6, 1939)

Potter, Van Rensselaer, M.S., Ph.D. McArdle Memorial Laboratory, University of Wisconsin Medical School, Madison. *Assistant Professor of Oncology.* (2, 1941)

Povitzky, Olga R., M.D., D.P.H. 235 E. 22nd St., New York City. *Bacteriologist, Bureau of Laboratories, New York City Department of Health.* (6, 1920)

Powell, Horace M., Sc.D. 5565 Washington Blvd., Indianapolis, Ind. *Bacteriologist, Eli Lilly & Co.* (6, 1934)

Power, Marschelle H., M.S., Ph.D. Mayo Clinic, Rochester, Minn. *Associate Professor of Physiological Chemistry, Mayo Foundation, University of Minnesota.* (2, 1932)

Pratt, Frederick H., A.M., M.D. 80 E. Concord St., Boston, Mass. *Professor of Physiology, Boston University School of Medicine.* (1, 1919)

Pratt, Joseph H., A.M., M.D. New England Medical Center, 25 Bennet St., Boston, Mass. *Physician-in-Chief, Boston Dispensary, and Joseph H. Pratt Diagnostic Clinic; Professor of Clinical Medicine, Tufts Medical School.* (1, 1910; 3, 1910; 4, 1927)

Preisler, Paul W., M.S., Ph.D. Station Hospital, Fort Sam Houston, Texas. *Captain, Sanitary Corps, U. S. A. (On leave of absence from Washington University Medical School, St. Louis, Mo.).* (2, 1931)

Prinzmetal, Myron, M.A., M.D. 2007 Wilshire Blvd., Los Angeles, Calif. *Instructor in Medicine and Lecturer in Physiology, University of Southern California Medical School.* (3, 1941)

Prosser, C. Ladd, Ph.D. University of Illinois, Urbana. *Assistant Professor of Zoology.* (1, 1935)

Pucher, George W., Ph.D. Connecticut Agricultural Experiment Station, New Haven. *Research Associate.* (2, 1927)

Puestow, Charles B., M.D., M.S., Ph.D. University of Illinois, College of Medicine, 1853 W. Polk St., Chicago. *Assistant Professor of Surgery.* (1, 1934)

Pugsley, Leonard I., Ph.D. Department of Pensions and National Health, Laboratory of Hygiene, Ottawa, Canada. *Pharmacologist.* (2, 1937)

Queen, Frank B., M.D. Passavant Memorial Hospital, Chicago, Ill. *Assistant Professor of Pathology, Northwestern University Medical School; Assistant Director, Patterson Cancer Clinic of Northwestern University Medical School, and Director, Patterson Laboratory for Cancer, Research, Passavant Memorial Hospital; Director of Laboratories, Passavant Memorial Hospital.* (4, 1941)

Quick, Armand J., M.D., Ph.D. 561 N. 15th St., Milwaukee, Wis. *Associate Professor of Pharmacology, Marquette Medical School.* (2, 1932; 3, 1937)

Quigley, J. P., Ph.D. Western Reserve University, Cleveland, O. *Associate Professor of Physiology.* (1, 1929)

Quinby, William Carter, M.D. Peter Bent Brigham Hospital, Boston, Mass. *Clinical Professor of Genito-urinary Surgery, Harvard Medical School.* (1, 1916)

Quinn, Edmond John, Ph.D. 106 N. Lee Ave., Rockville Center, Long Island, N. Y. *Medicinal Sales Division, Merck & Co., Inc., Rahway, N. J.* (2, 1927; 5, 1933)

Rabinowitch, I. M., D.Sc., M.D., C.M., F.R.C.P., F.A.C.P. The Montreal General Hospital, Montreal, Canada. *Associate Professor of Medicine and Lecturer in Biochemistry, McGill University; Director, Department of Metabolism, Montreal General Hospital.* (2, 1928)

Rackemann, Francis M., M.D. 263 Beacon St., Boston, Mass. *Physician, Massachusetts General Hospital; Lecturer in Medicine, Harvard Medical School.* (6, 1923)

Raffel, Sidney, Sc.D., M.D. Department of Bacteriology and Experimental Pathology, Stanford University, Calif. *Assistant Professor.* (6, 1938)

Raiziss, George W., Ph.D. 1720 Lombard St., Philadelphia, Pa. *Professor of Chemotherapy, Graduate School of Medicine, and Director, Dermatological Research Laboratory, University of Pennsylvania.* (2, 1913)

Rake, Geoffrey W., M.B., M.R.C.S., L.R.C.P. Division of Microbiology, The Squibb Institute for Medical Research, New Brunswick, N. J. *Head, Division of Microbiology.* (6, 1939)

Rakestraw, Norris W., A.M., Ph.D. Brown University, Providence, R. I. *Associate Professor of Chemistry.* (2, 1925)

Rakieten, Nathan, Ph.D. 4 Hillhouse Ave., New Haven, Conn. *Instructor in Applied Physiology, Yale University.* (1, 1941)

Ralli, Elaine P., M.D. 477 First Ave., New York City. *Associate Professor of Medicine, New York University College of Medicine.* (1, 1934; 5, 1933)

Ramsey, Robert Weberg, M.S., Ph.D. School of Medicine and Dentistry, University of Rochester, Rochester, N. Y. *Associate in Physiology.* (1, 1939)

Randall, Lowell O., Ph.D. Burroughs Wellcome Co., Tuckahoe, N. Y. *Physiologist.* (2, 1939)

Rane, Leo, Ph.D. Lederle Laboratories, Inc., Pearl River, N. Y. *Department Head, Normal Blood Plasma.* (6, 1942)

Rapoport, Samuel, M.D., Ph.D. The Children's Hospital Research Foundation, Elland and Bethesda, Cincinnati, O. *Research Associate.* (2, 1941)

Rapport, David, M.D. 416 Huntington Ave., Boston, Mass. *Professor of Physiology, Tufts College Medical School.* (1, 1922)

Rasmussen, Andrew Theodore, Ph.D. University of Minnesota Medical School, Minneapolis. *Professor of Neurology.* (1, 1919)

Ratner, Bret, M.D. New York University College of Medicine, 50 E. 78th St., New York City. *Professor of Pediatrics.* (4, 1940; 6, 1928)

Raulston, B. O., M.D. 200 S. Hudson Ave., Los Angeles, Calif. *Professor of Medicine, Director of Clinical Teaching, and Associate Dean, the University of Southern California, School of Medicine.* (3, 1942)

Ravdin, I. S., M.D. University of Pennsylvania School of Medicine, Philadelphia. *Harrison Professor of Surgery; Surgeon, Hospital of the University of Pennsylvania.* (1, 1930; 4, 1930)

Ray, George B., A.M., Ph.D. Long Island College of Medicine, 350 Henry St., Brooklyn, N. Y. *Professor of Physiology and Pharmacology.* (1, 1924)

Raymond, Albert L., Ph.D. 4737 Ravenwood Ave., Chicago, Ill. *Director of Research, G. D. Searle & Co.* (2, 1932)

Redfield, Alfred C., Ph.D. Woods Hole, Mass. *Professor of Physiology, Harvard University.* (1, 1919)

Reed, Carlos Isaac, A.M., Ph.D. College of Medicine, University of Illinois, 1853 W. Polk St., Chicago. *Professor of Physiology.* (1, 1923)

Reed, Howard S., Ph.D. 3048 Life Sciences Bldg., University of California, Berkeley. *Professor of Plant Physiology.* (2, 1909)

Rees, Maurice Holmes, A.M., Ph.D., M.D. University of Colorado School of Medicine, Denver. *Professor of Physiology and Pharmacology; Dean of the University of Colorado School of Medicine and Hospitals.* (1, 1922)

Reid, Marion Adelaide, A.M., Ph.D. 80 E. Concord St., Boston, Mass. *Instructor in Physiology, Boston University.* (1, 1941)

Reimann, Hobart A., M.D. Jefferson Hospital, Philadelphia, Pa. *Professor of Medicine, Jefferson Medical College.* (4, 1933)

Reimann, Stanley P., M.D., Sc.D. 703 W. Phil-Ellena St., Mount Airy, Philadelphia, Pa. *Director of the Research Institute of the Lankenau Hospital; Associate Professor of Surgical Pathology, Graduate School of Medicine, University of Pennsylvania; Professor of Oncology, Hahnemann Medical College and Hospital, Philadelphia.* (1, 1921; 4, 1924)

Reiner, Laszlo, M.D., Ph.D. Wallace & Tiernan Co., Inc. Research Division, Belleville, N. J. (2, 1942; 6, 1933)

Reinhold, John G., M.S., Ph.D. Philadelphia General Hospital, 34th St. and Curie Ave., Philadelphia, Pa. *Chief Biochemist; Instructor in Physiological Chemistry, University of Pennsylvania.* (2, 1936)

Remington, Roe E., M.A., Ph.D., D.Sc. Medical College of South Carolina, Charleston. *Professor of Nutrition and Director of Food Research Laboratory.* (2, 1930; 5, 1934)

Renfrew, Alice G., Ph.D. Mellon Institute of Industrial Research, University of Pittsburgh, Pittsburgh, Pa. *Fellow, Department of Research in Pure Chemistry.* (2, 1939)

Renshaw, Birdsey, M.A., Ph.D. Oberlin College, Oberlin, O. *Assistant Professor of Physiology.* (1, 1941)

Reynolds, Chapman, M.D. Louisiana State University, New Orleans. *Assistant Professor of Pharmacology.* (3, 1937)

Reynolds, Samuel R. M., Ph.D. Department of Embryology, Carnegie Institution of Washington, Wolfe and Madison Sts., Baltimore, Md. *Research Associate.* (1, 1932)

Reznikoff, Paul, M.D. New York Hospital, 525 E. 68th St., New York City. *Associate Professor of Clinical Medicine, Cornell University Medical College.* (1, 1927)

Rhoads, Cornelius Packard, M.D. Memorial Hospital, 444 E. 68th St., New York City. *Director.* (4, 1930)

Rice, James C., A.M., Ph.D. Louisiana State University School of Medicine, 1542 Tulane Ave., New Orleans. *Instructor in Pharmacology.* (3, 1941)

Rich, Arnold Rice, M.D. Johns Hopkins Hospital, Baltimore, Md. *Associate Professor of Pathology, Johns Hopkins University.* (4, 1924)

Rice, Christine E., M.A., Ph.D. 616 Madison Ave., Albany, N. Y. *Assistant Bacteriologist, Division of Laboratories and Research, New York State Department of Health.* (6, 1938)

Richards, Alfred N., A.M., Ph.D., Sc.D., M.D. (hon.), LL.D. University of Pennsylvania Medical School, Philadelphia. *Professor of Pharmacology and Vice-President in Charge of Medical Affairs; Member, National Academy of Sciences.* (1, 1900; 2, 1906; 3, 1909)

Richards, Oscar W., M.A., Ph.D. Research Department, Spencer Lens Co., 19 Doat St., Buffalo, N. Y. *Research Biologist.* (1, 1934)

Richards, Richard Kohn, M.D. Abbott Laboratories, North Chicago, Ill. *Chief Pharmacologist.* (1, 1938)

Richardson, Arthur P., M.D. University of Tennessee Medical School, Memphis. *Associate Professor and Head, Department of Pharmacology.* (3, 1939)

Richardson, Luther R., Ph.D. University of Missouri, Columbia. *Instructor in Agricultural Chemistry.* (5, 1942)

Richter, Curt P., Ph.D. Phipps Psychiatric Clinic, The Johns Hopkins Hospital, Baltimore, Md. *Associate Professor of Psycho-biology, Johns Hopkins University.* (1, 1924)

Richter, Maurice N., M.D. 303 E. 20th St., New York City. *Professor of Pathology, Columbia University, New York Post-Graduate Medical School; Director, Department of Pathology, New York Post-Graduate Medical School and Hospital.* (4, 1931)

Ricketts, Henry T., M.D. University of Chicago, Chicago, Ill. *Assistant Professor of Medicine.* (1, 1940)

Riddle, Oscar, Ph.D. Cold Spring Harbor, L. I., N. Y. *Resident Staff, Carnegie Station for Experimental Evolution; Member of the National Academy of Sciences.* (1, 1919)

Riegel, Byron, A.M., Ph.D. Department of Chemistry, Northwestern University, Evanston, Ill. *Assistant Professor.* (2, 1942)

Riegel, Cecilia, M.S., Ph.D. Room 563, University Hospital, Philadelphia, Pa. *Research Associate, Department of Research Surgery, University of Pennsylvania School of Medicine.* (2, 1938)

Ries, Ferd A., M.D. 300 E. North Ave., Baltimore, Md. *Instructor in Neurology, Johns Hopkins University.* (1, 1933)

Rigdon, R. H., M.D. Pathological Institute, The University of Tennessee, Memphis. *Associate Professor of Pathology.* (4, 1941)

Riggs, Lloyd K., Ph.D. % Kraft Cheese Co., 500 Peshtigo Court, Chicago, Ill. *Director of Research.* (2, 1929)

Rinehart, James F., M.D. University of California Medical School, Parnassus and Third Aves., San Francisco. *Professor of Pathology and Medicine.* (4, 1933)

Ring, Gordon C., M.A., Ph.D. Ohio State University, Columbus. *Assistant Professor of Physiology.* (1, 1933)

Rioch, David McKenzie, M.D. Washington University School of Medicine, St. Louis, Mo.

Professor of Neurology and Head of the Department of Neuropsychiatry. (1, 1931)

Rittenberg, David, Ph.D. 630 W. 168th St., New York City. *Assistant Professor, College of Physicians and Surgeons, Columbia University.* (2, 1939)

Ritzman, E. G., A.M., Science (hon.). University of New Hampshire, Durham. *Research Professor.* (5, 1933)

Rivers, T. M., M.D., Sc.D. The Hospital of the Rockefeller Institute for Medical Research, 66th St. and York Ave., New York City. *Director of the Hospital; Member of the National Academy of Sciences.* (4, 1925; 6, 1921)

Robb, Jane Sands, Sc.D., M.D. College of Medicine, Syracuse University, 761 Irving Ave., Syracuse, N. Y. *Associate Professor of Pharmacology.* (1, 1924)

Robbins, Benjamin Howard, M.S., M.D. Vanderbilt Univ. School of Medicine, Nashville, Tenn. *Associate Professor of Pharmacology.* (3, 1936)

Roberts, Lydia J., Ph.D. University of Chicago, Chicago, Ill. *Professor and Chairman of Department of Home Economics.* (5, 1933)

Robertson, Elizabeth Chant, M.D., M.A., Ph.D. University of Toronto, Toronto, Canada. *Research Fellow in Paediatrics.* (5, 1939)

Robertson, Oswald H., M.D. University of Chicago, Chicago, Ill. *Professor of Medicine.* (4, 1932)

Robinson, Charles Summers, Ph.D. Medical School, Vanderbilt University, Nashville, Tenn. *Professor of Biochemistry.* (2, 1925)

Robinson, Elliott S., M.D., Ph.D. 5 Oakwood Terrace, Newton Centre, Mass. *Lieutenant Colonel, M. C., U. S. A.* (6, 1935)

Robinson, G. Canby, M.D., Sc.D., LL.D. Johns Hopkins Hospital, Baltimore, Md. *Lecturer in Medicine, Johns Hopkins University.* (1, 1912; 3, 1921)

Robinson, George Henry, Ph.D. 320 E. North Ave., N. S., Pittsburgh, Pa. *Bacteriologist, Wm. H. Singer Research Laboratory and Allegheny General Hospital; Lecturer in Bacteriology, University of Pittsburgh School of Medicine.* (4, 1930)

Robinson, Howard West, Ph.D. Children's Hospital Research Foundation, Cincinnati, O. *Fellow; Assistant Professor, Research Pediatrics, College of Medicine; Assistant Professor of Biochemistry, Graduate School, University of Cincinnati.* (2, 1929)

Robinson, Sid, Ph.D. Fatigue Laboratory, Morgan Hall, Soldiers Field Station, Boston, Mass. (1, 1941)

Robscheit-Robbins, F. S., Ph.D. University of Rochester School of Medicine and Dentistry, Rochester, N. Y. *Associate in Pathology.* (1, 1925; 4, 1930)

Rodbard, Simon, Ph.D. Michael Reese Hospital, Chicago, Ill. *Research Associate, Cardiovascular Dept.* (1, 1942)

Roe, Joseph Hyram, M.A., Ph.D. George Washington University School of Medicine, Washington, D. C. *Professor of Biochemistry.* (2, 1927; 5, 1933)

Roeder, Kenneth D., M.A. Tufts College, Medford, Mass. *Assistant Professor of Biology.* (1, 1942)

Roepke, Martin Henry, Ph.D. University Farm, St. Paul, Minn. *Professor, Veterinary Medicine.* (3, 1937)

Rogers, Charles G., A.M., Ph.D., Sc.D. Oberlin College, Oberlin, O. *Professor of Comparative Physiology.* (1, 1911)

Rogers, Fred T., A.M., Ph.D., M.D. Dallas Medical and Surgical Clinic, Dallas, Texas. (1, 1917)

Rogoff, Julius M., Ph.G., M.D., Sc.D. School of Medicine, University of Pittsburgh, Pittsburgh, Pa. *Professor of Endocrinology.* (1, 1916; 3, 1916)

Ronzoni, Ethel, M.A., Ph.D. Washington University Medical School, St. Louis, Mo. *Assistant Professor of Biological Chemistry.* (2, 1923)

Root, Howard F., M.D. 44 Dwight St., Brookline, Mass. *Instructor in Medicine, Harvard Medical School.* (5, 1933)

Root, Walter S., Ph.D. College of Physicians and Surgeons, Columbia University, 630 W. 168th St., New York City. *Associate Professor of Physiology.* (1, 1932)

Rosahn, Paul D., M.D. Yale University School of Medicine, New Haven, Conn. *Assistant Clinical Professor of Pathology.* (4, 1934)

Rose, Anton Richard, M.S., Ph.D. Box 176, Edgewater, N. J. *Biochemist, Prudential Insurance Company of America.* (2, 1916; 5, 1933)

Rose, William C., Ph.D. University of Illinois, Urbana. *Professor of Biochemistry; Member, National Academy of Sciences.* (2, 1912; 5, 1933)

Rosenblueth, Arturo, M.D. Harvard Medical School, Boston, Mass. *Assistant Professor of Physiology.* (1, 1932)

Rosenfeld, Morris, M.D. Johns Hopkins School of Medicine, Baltimore, Md. *Associate in Pharmacology and Experimental Therapeutics.* (3, 1934)

Rosenow, Edward C., M.D., hon. LL.D. and D.Sc. Mayo Clinic, Rochester, Minn. *Professor of Experimental Bacteriology, Mayo Foundation, University of Minnesota.* (4, prior to 1920; (6, 1915)

Rosenthal, Sanford M., M.D. National Institute of Health, Washington, D. C. *Senior Pharmacologist, U. S. Public Health Service.* (3, 1925)

Rosenthal, S. R., M.D., Ph.D. University of Illinois College of Medicine, Chicago. *Assistant Professor of Bacteriology and Public Health in Dept. of Pathology and Bacteriology; Director, Tice Laboratory for B. C. G. Vaccination against Tuberculosis, Municipal Tuberculosis Sanatorium.* (4, 1941)

Ross, Joseph F., M.D. The Robert Dawson Evans Memorial, 78 E. Concord St., Boston, Mass. *Instructor in Medicine, Boston University School of Medicine; Assistant Physician, Evans Memorial.* (4, 1941)

Ross, William F., Ph.D. 12 Oxford St., Cambridge, Mass. *Instructor, Department of Chemistry, Harvard University.* (2, 1940)

Roth, George B., M.D. 1335 H St., N.W., Washington, D. C. *Professor of Physiology and Pharmacology, George Washington University School of Medicine.* (1, 1914; 3, 1911)

Roth, Grace M., M.S., Ph.D. Mayo Clinic, Rochester, Minn. *Associate in Clinical Physiology.* (1, 1939)

Roth, Paul, M.D. Battle Creek Sanitarium, Battle Creek, Mich. *Director of Physical Therapy.* (1, 1929; 5, 1933)

Rothemund, Paul W. K., Dipl.-Ing., Dr.-Ing. (Munich). Antioch College, Yellow Springs, O. *Associate Professor of Biochemistry, and Research Chemist, The C. F. Kettering Foundation, Antioch College; Associate Professor (Non-resident), Department of Chemistry, Ohio State University.* (2, 1940)

Rous, Peyton, M.D., Sc.D. Rockefeller Institute for Medical Research, York Ave. at 66th St., New York City. *Member; Member of the National Academy of Sciences.* (4, prior to 1920)

Routh, Joseph I., M.S., Ph.D. Chemistry Department, State University of Iowa, Iowa City. *Instructor in Biochemistry.* (2, 1942)

Rowntree, Jennie I., M.S., Ph.D. University of Washington, Seattle. *Professor of Home Economics.* (5, 1933)

Rowntree, L. G., Sc.D., M.D., F.A.C.P. Philadelphia Institute for Medical Research, 34th and Pine Sts., Philadelphia, Pa. *Director; Research Clinician, Philadelphia General Hospital; Chief, Medical Selective Service National Headquarters, Washington, D. C.* (1, 1911; 2, 1910; 3, 1908; 4, prior to 1920)

Rubenstein, Boris B., M.A., M.D., Ph.D. Michael Reese Hospital, 29th St. and Ellis Ave., Chicago, Ill. *Research Associate.* (1, 1934)

Rubin, Morton A., Ph.D. (Capt., Signal Corps, U. S. Army). 720 21st St., South Arlington, Va. *Office of the Chief Signal Officer, Military Personnel Division, Washington, D. C.* (1, 1940)

Ruch, Theodore C., M.A., Ph.D. Yale University School of Medicine, New Haven, Conn. *Assistant Professor of Physiology.* (1, 1933)

Rusch, Harold Paul, M.D. University of Wisconsin, McArdle Memorial Laboratory, Madison. *Assistant Professor of Oncology; Bowman Cancer Research Fellow.* (4, 1940)

Russell, Jane A., Ph.D. Yale University School of Medicine, 333 Cedar St., New Haven, Conn. *Instructor in Physiological Chemistry.* (1, 1939)

Russell, Walter C., Ph.D. New Jersey Agricultural Experiment Station and Rutgers University, New Brunswick. *Biochemist in Nutrition and Professor of Agricultural Biochemistry.* (2, 1932; 5, 1933)

Ryan, Andrew Howard, M.D. 210 E. Ohio St., Suite 402, Chicago, Ill. (1, 1912)

Sabin, Florence R., M.D., Sc.D. 1333 E. 10th Ave., Denver, Colo. *Member Emeritus of the Rockefeller Institute; Member of the National Academy of Sciences.* (1, 1923)

Sachs, Ernest, M.D. 97 Arundel Pl., St. Louis, Mo. *Professor of Clinical Neurological Surgery, Washington University Medical School.* (1, 1910)

Sacks, Jacob, Ph.D., M.D. University of Michigan Medical School, Ann Arbor. *Assistant Professor of Pharmacology.* (3, 1933)

Sah, Peter P. T., M.S., Ph.D. Department of Chemistry, Fu Jen University, Peiping, China; *Professor of Chemistry; Lecturer in Pharmacology, Peiping Union Medical College.* (3, 1941)

Sahyun, Melville, A.M., Ph.D. Frederick Stearns & Co., 6533 E. Jefferson St., Detroit, Mich. *Director, Biochemical Research Division.* (2, 1932)

Salant, William, M.D. 812 Riverside Drive, New York City. (1, 1905; 2, 1906; 3, 1908)

Salmon, W. D., A.M. Alabama Polytechnic Institute, Auburn. *Animal Nutritionist.* (2, 1929; 5, 1933)

Salter, William T., M.D. Yale School of Medicine, 333 Cedar St., New Haven, Conn. *Professor of Pharmacology.* (1, 1933; 3, 1942; 5, 1934)

Sampson, John J., M.D. 490 Post St., San Francisco, Calif. *Assistant Clinical Professor of Medicine, University of California Medical School.* (1, 1932)

Sampson, Myra, A.M., Ph.D. Smith College, Northampton, Mass. *Professor and Chairman of Department of Zoology.* (5, 1935)

Samuels, Leo T., Ph.D. 311 Millard Hall, University of Minnesota, Minneapolis. *Assistant Professor of Physiological Chemistry.* (2, 1941; 3, 1937)

Sandels, Margaret R., A.M., Ph.D. Florida State College for Women, Tallahassee. *Dean of School of Home Economics; Professor of Nutrition.* (5, 1933)

Sandiford, Irene, Ph.D. Billings Hospital, University of Chicago, Chicago, Ill. *Assistant Professor of Medicine.* (2, 1925; 5, 1933)

Sanford, Arthur H., A.M., M.D. Clinical Laboratories, Mayo Clinic, Rochester, Minn. *Head, Division of Clinical Laboratories.* (6, 1920)

Santos, Francisco O., M.S., Ph.D. University of the Philippines, Los Banos, Laguna. *Professor and Head of Department of Agricultural Chemistry, College of Agriculture.* (5, 1936)

Saphir, Otto, M.D. Michael Reese Hospital, 29th St. and Ellis Ave., Chicago, Ill. *Pathologist, Michael Reese Hospital; Associate Professor of Pathology, University of Illinois Medical School.* (4, 1927)

Sappington, Samuel W., M.D., D.Sc. 235 N. 15th St., Philadelphia, Pa. *Professor of Pathology, Hahnemann Hospital.* (6, 1913)

Saslow, George, Ph.D., M.D. 72 Grozier Rd., Cambridge, Mass. *Assistant in Psychiatry, Harvard Medical School.* (1, 1936)

Satterfield, George H., A.M. University of North Carolina, Raleigh. *Professor of Biochemistry.* (5, 1941)

Saul, Leon Joseph, M.A., M.D. The Institute for Psychoanalysis, 43 E. Ohio St., Chicago, Ill. (1, 1933)

Saunders, Felix, Ph.D. 231 Playa del Sur, La Jolla, Calif. (2, 1938)

Sawyer, Margaret E. MacKay, M.A., Ph.D. 183 University Ave., Kingston, Ontario, Canada. (1, 1935)

Sawyer, Wilbur A., M.D. 29 Ferndale Drive, Hastings-on-Hudson, N. Y. *Director, International Health Division, Rockefeller Foundation.* (4, 1930; 6, 1935)

Scammon, Richard E., M.A., Ph.D. University of Minnesota, Minneapolis. *Distinguished Service Professor in the Graduate School.* (1, 1923)

Scharles, Frederick H., M.D. 1405 Bryant Bldg., Kansas City, Mo. (5, 1935)

Schattenberg, Herbert John, M.S., M.D. Medical and Surgical Memorial Hospital and Tumor Clinic, 215 Camden St., San Antonio, Texas. *Pathologist.* (4, 1940)

Schenken, John R., M.D. Louisiana State University School of Medicine, New Orleans. *Professor of Pathology and Bacteriology.* (4, 1942)

Scherp, Henry W., M.S., Ph.D. University of Rochester Medical School, 260 Crittenden Blvd., Rochester, N. Y. *Assistant Professor of Immunochemistry.* (6, 1940)

Schick, Bela, M.D. 17 E. 84th St., New York City. *Pediatrician, Mt. Sinai Hospital.* (6, 1924)

Schlenk, Fritz, Ph.D. University of Texas Medical School, Galveston. *Assistant Professor.* (2, 1942)

Schlesinger, M. J., Ph.D., M.D. Beth Israel Hospital, 330 Brookline Ave., Boston, Mass. *Associate in Pathology, Harvard Medical School; Director of Pathology, Beth Israel Hospital.* (4, 1942; 6, 1921)

Schlomovitz, Benjamin H., M.D. 1210 Majestic Bldg., 231 W. Wisconsin Ave., Milwaukee, Wis. *Director, Clinical and Research Laboratory, Veterans Administration Hospital, Wood, Wisconsin.* (1, 1919)

Schlutz, F. W., M.D. 920 E. 59th St., Chicago, Ill. *Chairman of Pediatric Department and Bobs Roberts Hospital, University of Chicago.* (2, 1924; 5, 1936)

Schmeisser, Harry C., M.D. University of Tennessee, Memphis. *Professor of Pathology and Bacteriology.* (4, 1937)

Schmidt, Carl F., M.D. Medical School, University of Pennsylvania, Philadelphia. *Professor of Pharmacology.* (1, 1929; 3, 1924)

Schmidt, Carl L. A., M.S., Ph.D. University of California, Berkeley. *Professor of Biochemistry; Chairman of Division; Dean of the College of Pharmacy.* (2, 1919)

Schmidt, C. Robert, Ph.D., M.D. Hertzler Clinic, Halstead, Kan. *Resident Surgeon.* (1, 1940)

Schmidt, Gerhard, M.D. Boston Dispensary, 25 Bennett St., Boston, Mass. (2, 1939)

Schmidt, Leon H., M.S., Ph.D. Christ Hospital, Institute for Medical Research, Cincinnati, O. *Director of Research; Assistant Professor of Biological Chemistry, College of Medicine, University of Cincinnati.* (2, 1936)

Schmitt, Francis Otto, Ph.D. Dept. of Biology and Public Health, Massachusetts Institute of Technology, Cambridge. *Professor of Biology.* (1, 1930)

Schnedorf, Jerome G., M.D., Ph.D. The University of Kansas School of Medicine, Kansas City. *Associate in Surgery.* (1, 1941)

Schneider, Edward C., Ph.D., Sc.D. 25 Gordon Place, Middletown, Conn. *University Professor of Biology, Wesleyan University.* (1, 1912; 2, 1912)

Schoenbach, Emanuel B., M.D. 1480 Jesup Ave., New York City. *Resident Physician, Mt. Sinai Hospital.* (6, 1941)

Schradieck, Constant E., M.D. 65 Hazard Ave., Providence, R. I. *Director, Pathological Department, Homeopathic Hospital of Rhode Island.* (6, 1921)

Schreiner, Oswald, M.S., Ph.D. Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C. *Chief, Division of Soil Fertility Investigations.* (2, 1908)

Schroeder, E. F., M.S., Ph.D. G. D. Searle & Co., P. O. Box 5110, Chicago, Ill. *Research Biochemist.* (2, 1938)

Schuck, Cecelia, Ph.D. Purdue University, Lafayette, Ind. *Professor of Nutrition, Department of Home Economics.* (5, 1941)

Schultz, Edwin William, M.D. 743 Cooksey Lane, Stanford University, Calif. *Professor of Bacteriology and Experimental Pathology.* (4, 1927; 6, 1928)

Schultz, W. H., Ph.D. Greenspring Ave. & Taney Rd., Baltimore, Md. (1, 1907; 3, 1909)

Schultze, Max O., Ph.D. Department of Chemistry, University of Pittsburgh, Pittsburgh, Pa. *Research Fellow, Buhl Foundation.* (2, 1938)

Schwartze, Erich W., M.D. 1225 Talbert St., S. E., Washington, D. C. (3, 1920)

Scott, David Alymer, M.A., Ph.D. Connaught Laboratories, University of Toronto, Toronto 5, Ontario, Canada. *Senior Research Chemist.* (2, 1935)

Scott, Ernest L., Ph.D. 64 South St., Bogota, N. J. *Associate Professor of Physiology Emeritus, Columbia University.* (1, 1914; 2, 1915)

Scott, Frederick Hughes, Ph.D., Sc.D., M.B. University of Minnesota, Minneapolis. *Professor of Physiology.* (1, 1908; 2, 1909)

Scott, John C., Ph.D. Hahnemann Medical College, Philadelphia, Pa. *Professor of Physiology and Head of the Department.* (1, 1936)

Scott, R. W., A.M., M.D. City Hospital, Cleveland, O. *Professor of Clinical Medicine, Western Reserve University; Physician-in-chief, Cleveland City Hospital.* (1, 1917; 3, 1917)

Scott, V. Brown, Ph.D., M.D. 5427 University, Chicago, Ill. (1, 1941)

Scott, W. J. Merle, M.D. University of Rochester Medical School, Rochester, N. Y. *Associate Professor of Surgery.* (4, 1925)

Scudi, John Vincent, Ph.D. Merck Institute for Therapeutic Research, Rahway, N. J. *Biochemist.* (2, 1942)

Seager, Lloyd D., M.S., M.D. University of Tennessee Medical School, 870 Union Ave., Memphis. *Instructor in Pharmacology.* (3, 1939)

Sealock, Robert R., Ph.D. Department of Vital Economics, University of Rochester Medical School, Crittenden Blvd., Rochester, N. Y. *Assistant Professor of Physiological Chemistry.* (2, 1940; 5, 1941)

Seastone, C. V., Jr., M.D. University of Wisconsin Medical School, Madison. *Associate Professor of Medical Bacteriology.* (6, 1939)

Sebrell, W. H., Jr., M.D. National Institute of Health, Bethesda, Md. *Chief, Division of Chemotherapy.* (2, 1938; 5, 1937)

Seecof, David P., M.D. 1970 Daly Ave., Bronx, New York City. (4, 1927)

Seegal, David, M.D. Welfare Island, New York City. *Director, Research and Clinical Service, First Division, Welfare Hospital; Associate Professor of Medicine, Columbia University.* (6, 1930)

Seegers, Walter H., M.S., Ph.D. College of Medicine, State University of Iowa, Iowa City. *Research Associate.* (2, 1941)

Seevers, Maurice Harrison, Ph.D., M.D. University of Michigan School of Medicine, Ann Arbor. *Professor of Pharmacology and Chairman of the Department.* (1, 1933; 3, 1930)

Seibert, Florence B., Ph.D., Sc.D., LL.D. Henry Phipps Institute, 7th and Lombard Sts., Philadelphia, Pa. *Associate Professor of Biochemistry, University of Pennsylvania.* (2, 1925)

Seidell, Atherton, M.S., Ph.D. 2301 Connecticut Ave., Washington, D. C. *Special Expert, National Institute of Health.* (2, 1924)

Seifter, Joseph, M.D. 2109 Adelbert Rd., Cleveland, O. *Assistant Professor of Pharmacology, Western Reserve University.* (3, 1940)

Sellards, Andrew Watson, M.D. Harvard Medical School, Boston, Mass. *Associate Professor of Tropical Medicine; Consultant in Tropical Medicine, Massachusetts General Hospital.* (4, 1923)

Selle, Wilber Arthur, Ph.D. Medical School, University of Texas, Galveston. *Associate Professor of Physiology.* (1, 1938)

Selye, Hans, M.D., Ph.D. Medical Building, McGill University, Montreal, Que., Canada. *Assistant Professor of Anatomy.* (1, 1934)

Sendroy, Julius, Jr., M.A., Ph.D. Mercy Hospital, 2537 Prairie Ave., Chicago, Ill. *Professor of Chemistry and Chairman of the Department of Experimental Medicine, Loyola University.* (2, 1928)

Sevag, M. G., Ph.D. Department of Bacteriology, University of Pennsylvania School of Medicine, Philadelphia. *Assistant Professor of Biochemistry in Bacteriology.* (6, 1941)

Sevringhaus, Elmer L., M.A., M.D. Wisconsin General Hospital, Madison. *Professor of Medicine, University of Wisconsin; Consultant in Clinical Chemistry, Wisconsin Psychiatric Institute; Chemist to Wisconsin General Hospital.* (2, 1923; 5, 1939)

Shaffer, Morris F., D. Phil. The Massachusetts Antitoxin and Vaccine Laboratory, Jamaica Plain. *Senior Bacteriologist; Associate in the Department of Bacteriology and Immunology, Harvard Medical School.* (4, 1929; 6, 1937)

Shaffer, Philip A., Ph.D. Washington University Medical School, St. Louis, Mo. *Professor of Biological Chemistry and Dean; Member of the National Academy of Sciences.* (1, 1906; 2, 1906; 5, 1935)

Shannon, James A., M.D., Ph.D. Welfare Hospital, Welfare Island, New York City. *Director of Research Service, Third (New York University) Medical Division, Welfare Hospital,*

Department of Medicine, New York University College of Medicine. (1, 1933)

Shapiro, Herbert, A.M., Ph.D. Hahnemann Medical College, Philadelphia, Pa. *Instructor in Physiology.* (1, 1937)

Sharpless, George R., D.Sc. Henry Ford Hospital, Detroit, Mich. *Associate in Nutrition Research.* (5, 1942)

Shaw, Myrtle, M.S., Ph.D. 11 S. Lake Ave., Albany, N. Y. *Senior Bacteriologist, Division of Laboratories and Research, New York State Department of Health.* (6, 1937)

Shear, Murray, Jr., Ph.D. National Cancer Institute, Bethesda, Md. *Principal Biochemist.* (2, 1930)

Sheard, Charles, A.M., Ph.D. Mayo Foundation, Rochester, Minn. *Chief of the Division of Physics and Biophysical Research and Professor of Physiological Optics and Biophysics, University of Minnesota.* (1, 1925)

Sheehan, Donal, M.D., D.Sc. New York University College of Medicine, First Ave., New York City. *Professor of Anatomy and Director of Anatomical Laboratories.* (1, 1938)

Sheppard, Fay, M.S. University of Oklahoma Medical School, Oklahoma City. *Instructor in Biochemistry.* (2, 1936)

Sherman, Henry C., A.M., Ph.D., Sc.D. Columbia University, New York City. *Mitchell Professor of Chemistry and Executive Officer of the Department of Chemistry; Member, National Academy of Sciences.* (1, 1923; 2,1906; 5, 1933)

Sherwin, Carl Paxson, Sc.D., M.D., D.P.H., LL.D. 40 E. 61st St., New York City. *Director of Metabolic Service, St. Vincent's Hospital; Associate Physician, French Hospital.* (1, 1919; 2, 1917)

Sherwood, Noble P., Ph.D., M.D. 1801 Indiana St., Lawrence, Kan. *Professor of Bacteriology, University of Kansas.* (6, 1928)

Sherwood, Thomas Cecil, M.A., Ph.D. 206 Service Memorial Institute, University of Wisconsin, Madison. (1, 1938)

Shimkin, Michael Boris, M.D. U. S. Public Health Service, National Cancer Institute, Bethesda, Md. *Passed Assistant Surgeon.* (4, 1940)

Shlaer, Simon, M.A., Ph.D. Columbia University, New York City. *Research Associate in Biophysics.* (1, 1938)

Shock, Nathan W., Ph.D. Unit on Gerontology, U. S. Public Health Service, Baltimore City Hospitals, Baltimore, Md. *Senior Psychophysiologist, U. S. Public Health Service, National Institute of Health, Bethesda, Md.* (1, 1942)

Shoemaker, Harold A., M.S., Ph.D. University of Oklahoma School of Medicine, Oklahoma City. *Assistant Dean; Professor of Pharmacology.* (3, 1941)

Shohl, Alfred T., M.D. 300 Longwood Ave., Boston, Mass. *Research Associate in Pediatrics, Harvard Medical School.* (2, 1922; 5, 1933)

Shope, Richard E., M.D. Department of Animal and Plant Pathology, The Rockefeller Institute, Princeton, N. J. *Member.* (4, 1934)

Shorr, Ephraim, M.D. The New York Hospital, 525 East 68th St., New York City. *Assistant Professor of Medicine, Cornell University Medical College; Assistant Attending Physician, The New York Hospital.* (1, 1931; 3, 1942)

Shwartzman, Gregory, M.D. 230 E. 50th St., New York City. *Head of Department of Bacteriology, Mount Sinai Hospital; Clinical Professor of Bacteriology, Columbia University.* (4, 1929)

Sichel, F. J. M., Sc.M., Ph.D. College of Medicine, University of Vermont, Burlington. *Instructor in Physiology.* (1, 1939)

Sickles, Grace M., B.A. 2201 Twelfth St., Troy, N. Y. *Associate Bacteriologist, Division of Laboratories and Research, New York State Department of Health.* (6, 1932)

Sickles, Gretchen R., A.B. Division of Laboratories and Research, New York State Department of Health, Albany, N. Y. *Assistant Bacteriologist.* (6, 1937)

Siebert, Walter J., M.D. DePaul Hospital, St. Louis, Mo. *Director of Laboratories and Pathologist of DePaul and Lutheran Hospitals, St. Louis, and of St. Joseph Hospital, Alton, Ill.* (4, 1932)

Silvette, Herbert, M.S., Ph.D. University of Virginia Medical School, University. *Assistant Professor of Pharmacology.* (1, 1933; 3, 1940)

Simon, Frank A., M.D. 812 Heyburn Bldg., Louisville, Ky. (6, 1934)

Simonds, James P., Ph.D., M.D. Northwestern University Medical School, 303 E. Chicago Ave., Chicago, Ill. *Professor of Pathology.* (4, prior to 1920)

Simonson, Ernst, M.D. Mount Sinai Hospital, 12th and Kilbourn Ave., Milwaukee, Wis. *Research Fellow.* (1, 1941)

Sinclair, Robert Gordon, Ph.D. Queen's University, Kingston, Ont., Canada. *Professor of Biochemistry.* (2, 1931)

Slaughter, Donald, M.D. University of Vermont Medical School, Burlington. *Professor of Pharmacology and Physiology.* (3, 1938)

Slonaker, James R., Ph.D. 334 Kingsley Ave., Palo Alto, Calif. *Professor of Physiology, Leland Stanford Junior University.* (1, 1917)

Smadel, Joseph Edwin, M.D. Hospital of The Rockefeller Institute for Medical Research, 66th St. and York Ave., New York City. *Asso-*

ciate Member; Assistant Physician, Rockefeller Hospital. (4, 1940; 6, 1937)

Small, James C., M.D. 133 S. 36th St., Philadelphia, Pa. *Instructor in Medicine, Graduate School of Medicine, University of Pennsylvania.* (4, 1927)

Smetana, Hans, M.D. College of Physicians and Surgeons, 630 W. 168th St., New York City. *Assistant Professor of Pathology.* (4, 1934)

Smith, Arthur H., M.S., Ph.D. Wayne University College of Medicine, Detroit, Mich. *Professor of Physiological Chemistry.* (1, 1923; 2, 1921; 5, 1933)

Smith, Austin Edward, M.D., C.M., M.Sc.(Med.). American Medical Association, 535 N. Dearborn St., Chicago, Ill. *Acting Secretary of the Council on Pharmacy and Chemistry, American Medical Association; Research Associate (Instructor) Dept. of Pharmacology, University of Chicago.* (3, 1942)

Smith, Clarence A., M.S., Ph.D. Standard Brands, Inc., 595 Madison Ave., New York City. *Technical Director, Special Products Department.* (1, 1921)

Smith, Dietrich Conrad, A.M., Ph.D. University of Maryland School of Medicine, Lombard and Greene Sts., Baltimore. *Associate Professor of Physiology.* (1, 1937)

Smith, Elinor Van Dorn, Ph.D. 5 Middle St., Hadley, Mass. *Assistant Professor of Bacteriology, Smith College.* (6, 1940)

Smith, Elizabeth R. B., Ph.D. % Capt. Paul K. Smith, School of Aviation Medicine, Randolph Field, Texas. (2, 1938)

Smith, Erma A., A.M., Ph.D., M.D. Iowa State College, Ames. *Associate Professor of Physiology.* (1, 1928)

Smith, Fred M., M.D. State University of Iowa, Iowa City. *Professor of the Theory and Practice of Medicine and Head of the Department.* (1, 1925)

Smith, George H., M.A., Ph.D., M.A.(hon.), Sc.D. School of Medicine, Yale University, New Haven, Conn. *Professor of Immunology and Assistant Dean; Chairman, Department of Bacteriology, Yale University.* (6, 1918)

Smith, H. P., M.S., M.D. College of Medicine, State University of Iowa, Iowa City. *Professor of Pathology.* (1, 1937; 4, 1925)

Smith, Homer W., M.S., Sc.D. 477 First Ave., New York City. *Professor of Physiology, New York University College of Medicine.* (1, 1923; 2, 1930)

Smith, Lawrence Weld, M.D. Temple University School of Medicine, N. Broad St., Philadelphia, Pa. *Professor and Head of Department of Pathology; Director of Laboratories, Temple University Hospital.* (4, 1927)

Smith, Lee Irvin, A.M., Ph.D. School of Chemistry, University of Minnesota, Minneapolis. *Professor and Chief, Division of Organic Chemistry.* (2, 1942)

Smith, Margaret Cammack, A.M., Ph.D. University of Arizona, Tucson. *Professor of Nutrition; Nutrition Chemist, Agricultural Experiment Station, School of Home Economics.* (2, 1935; 5, 1933)

Smith, Maurice I., M.D. National Institute of Health, Bethesda, Md. *Principal Pharmacologist., U. S. Public Health Service.* (1, 1920; 3, 1916)

Smith, Paul Kenneth, Ph.D. School of Aviation Medicine, Randolph Field, Texas. *Director, Laboratory of Pharmacology. Captain, U. S. Air Corps; Assistant Professor of Pharmacology and Toxicology, Yale University.* (2, 1937; 3, 1937)

Smith, Paul W., M.S., Ph.D. School of Medicine, University of Oklahoma, 801 E. 13th St., Oklahoma City. *Assistant Professor of Pharmacology.* (1, 1933)

Smith, Philip Edward, M.S., Ph.D. 630 W. 168th St., New York City. *Professor of Anatomy, Columbia University; Member of the National Academy of Sciences.* (1, 1923)

Smith, Ralph G., M.D., Ph.D. Pharmacology Building, University of Michigan, Ann Arbor. *Associate Professor of Pharmacology.* (3, 1929)

Smith, Susan Gower, M.A. Duke University, Durham, N. C. *Associate, Department of Medicine and Nutrition, School of Medicine.* (5, 1939)

Smith, Sybil L., A.M. Principal Experiment Station Administrator, Office of Experiment Stations, U.S.D.A., Washington, D. C. (5, 1940)

Smith, Wilbur Kenneth, M.D. University of Rochester School of Medicine and Dentistry, 260 Crittenden Blvd., Rochester, N. Y. *Associate Professor of Anatomy.* (1, 1939)

Smith, Willie W., M.A., Ph.D. Alfred I. du Pont Institute of the Nemours Foundation, Wilmington, Del. (1, 1941)

Smithburn, Kenneth C., M.D. Yellow Fever Research Institute, P. O. Box 49, Entebee, Uganda, British East Africa. *Staff Member, International Health Division of The Rockefeller Foundation.* (6, 1937)

Smythe, C. V., M.S., Ph.D. University of Pittsburgh, Pittsburgh, Pa. *Research Fellow, Buhl Foundation; Assistant Professor of Chemistry.* (2, 1934)

Snell, Albert M., M.D. Mayo Clinic, Rochester, Minn. *Head of Section on Medicine at Mayo Clinic; Professor in Medicine, Mayo Foundation Graduate School, University of Minnesota.* (4, 1930)

Snell, Esmond E., M.A., Ph.D. Department of Chemistry, University of Texas, Austin. *As-*

sistant Professor of Chemistry and Research Biochemist. (2, 1942)

Snyder, Charles D., M.S., Ph.D. Johns Hopkins University School of Medicine, Baltimore, Md. *Professor Emeritus of Experimental Physiology.* (1, 1907)

Snyder, Franklin Faust, M.D. University of Chicago, Ill. *Associate, Department of Obstetrics.* (1, 1936)

Sobel, Albert E., Ch.E., M.A., Ph.D. Jewish Hospital of Brooklyn, Prospect Place and Classon Ave., Brooklyn, N. Y. *Director of Chemical Laboratories.* (2, 1939)

Sobotka, Harry H., Ph.D. Mount Sinai Hospital, Fifth Ave. and 100th St., New York City. *Head, Department of Chemistry.* (2, 1932; 5, 1933)

Solandt, Donald Young, M.A., M.D., Ph.D. University of Toronto, Toronto, Ont., Canada. *Associate Professor of Physiology; Head of the Department of Physiological Hygiene.* (1, 1937)

Sollmann, Torald, M.D., Sc.D. School of Medicine, Western Reserve University, 2109 Adelbert Rd., Cleveland, O. *Dean and Professor of Pharmacology and Materia Medica.* (1, 1902; 2, 1906; 3, 1908)

Somogyi, Michael, Ph.D. 216 S. Kingshighway, St. Louis, Mo. *Biochemist, Jewish Hospital of St. Louis.* (2, 1927)

Soskin, Samuel, M.D., M.A., Ph.D. Michael Reese Hospital, Chicago, Ill. *Director of Metabolic and Endocrine Research; Professoral Lecturer in Physiology, University of Chicago.* (1, 1930; 5, 1933)

Soule, Malcolm H., Sc.D., LL.D. University of Michigan, Ann Arbor. *Professor of Bacteriology, Director of the Hygienic Laboratory and Chairman of the Department of Bacteriology.* (4, 1927; 6, 1925)

Spain, Will C., M.D. 116 E. 53rd St., New York City. *Clinical Professor of Medicine, Post-Graduate Medical School, Columbia University.* (6, 1923)

Spealman, C. R., M.A., Ph.D. Medical College of Virginia, Richmond. *Associate in Physiology and Pharmacology.* (1, 1940)

Specht, Heinz, Ph.D. National Institute of Health, Rockville Pike, Bethesda, Md. *Associate Research Physiologist.* (1, 1941)

Spencer, Henry James, M.A., M.D. Bellevue Hospital, 24 W. 10th St., New York City. *Director Second Medical Division. Associate Professor of Clinical Medicine, Cornell University Medical College.* (5, 1935)

Sperry, Warren M., M.S., Ph.D. 722 W. 168th St., New York City. *Principal Research Biochemist, New York State Psychiatric Institute and Hospital; Assistant Professor of Biological Chemistry, Columbia University.* (2, 1929; 5, 1933)

Spiegel, Ernest A., M.D. Temple University School of Medicine, Broad and Ontario Sts., Philadelphia, Pa. *Professor of Experimental Neurology.* (1, 1936)

Spiegel-Adolf, Mona, M.D. Temple University School of Medicine, Broad St. at Ontario Ave., Philadelphia, Pa. *Professor and Head of Department of Colloid Chemistry.* (2, 1933)

Spies, Tom D., M.D. Feb.-Nov. Hillman Hospital, Birmingham, Ala. Nov.-Feb. General Hospital, Cincinnati, O. *Associate Professor of Medicine, Univ. of Cincinnati College of Medicine. Visiting Professor of Medical Research, Univ. of Alabama School of Medicine. Professor of Medical Research, Univ. of Texas School of Medicine. Director, Nutrition Clinic, Hillman Hospital, Birmingham, Ala.* (3, 1941; 4, 1940; 5, 1938)

Spink, Wesley W., M.D. University of Minnesota Hospital, Minneapolis. *Associate Professor of Medicine, University of Minnesota Medical School.* (3, 1940; 4, 1940; 6, 1940)

Spohn, Adelaide, M.S., Ph.D. Elizabeth McCormick Memorial Fund, 848 N. Dearborn St., Chicago, Ill. (5, 1933)

Sproul, Edith E., M.D. Columbia University, College of Physicians and Surgeons, New York City. *Assistant Professor of Pathology.* (4, 1941)

Sprunt, Douglas H., M.D., M.S. Box 3611, Duke Hospital, Durham, N. C. *Associate Professor of Pathology.* (4, 1934; 6, 1936)

Stadie, William C., M.D. 821 Maloney Clinic, 36th and Spruce Sts., Philadelphia, Pa. *Professor of Research Medicine, University of Pennsylvania.* (2, 1922)

Stainsby, Wendell J., M.D., C.M. Geisinger Memorial Hospital, Danville, Pa. *Chief Physician.* (6, 1930)

Stanley, Wendell M., M.S., Ph.D., Sc.D. Rockefeller Institute for Medical Research, Princeton, N. J. *Member; Member National Academy of Sciences.* (2, 1936)

Stannard, James Newell, Ph.D. National Institute of Health, Division of Industrial Hygiene, Bethesda, Md. *Pharmacologist.* (1, 1938)

Stare, Fredrick J., Ph.D., M.D. Department of Biological Chemistry, Harvard Medical School, Boston, Mass. *Assistant Professor of Nutrition.* (2, 1937; 5, 1942)

Starr, Isaac, M.D. 817 Maloney Clinic, University Hospital, Philadelphia, Pa. *Hartzell Professor of Research Therapeutics, University of Pennsylvania.* (1, 1929; 3, 1942)

Stavraky, George W., M.D., C.M., M.Sc. Medical School, University of Western Ontario,

London, Ont., Canada. *Assistant Professor of Physiology.* (1, 1937)

Stearns, Genevieve, M.S., Ph.D. College of Medicine, State University of Iowa, Iowa City. *Research Associate Professor of Pediatrics.* (2, 1932; 5, 1937)

Steel, Matthew, Ph.D. Long Island College of Medicine, 350 Henry St., Brooklyn, N. Y. *Professor of Biological Chemistry.* (2, 1909)

Steele, J. Murray, M.D. Welfare Hospital, Welfare Island, New York City. *Associate Professor of Medicine, New York University; Director 3rd (New York University) Medical Division of Welfare Hospital.* (1, 1936)

Steenbock, Harry, M.S., Ph.D., Sc.D. University of Wisconsin, Madison. *Professor of Biochemistry.* (2, 1912; 5, 1933)

Steggerda, F. R., M.A., Ph.D. 416 Natural History Building, University of Illinois, Urbana. *Assistant Professor of Physiology.* (1, 1934)

Stehle, Raymond Louis, A.M., Ph.D. Faculty of Medicine, McGill University, Montreal, Canada. *Professor of Pharmacology.* (2, 1920; 3, 1922)

Steigmann, Frederick, M.S., M.D. 348 S. Hamlin Ave., Chicago, Ill. *Associate in Medicine, College of Medicine, University of Illinois; Associate Attending Physician, Cook County Hospital.* (3, 1942)

Steiman, S. E., M.A., Ph.D. 364 Riverway, Boston, Mass. (1, 1939)

Steinbach, H. Burr, M.A., Ph.D. Washington University, St. Louis, Mo. *Associate Professor of Zoology.* (1, 1934)

Steinberg, Bernhard, M.D. Toledo Hospital Institute of Medical Research, Toledo, O. *Director of the Institute of Medical Research and of the Clinical Laboratories of the Toledo Hospital.* (4, 1928)

Steiner, Paul E., M.D. The University of Chicago, Chicago, Ill. *Associate Professor of Pathology.* (4, 1939)

Steinhardt, Jacinto, A.M., Ph.D. 1548 East-West Highway, Silver Springs, Md. *Research Associate, Division of War Research, Columbia University, New York City.* (2, 1939)

Steinhaus, Arthur H., M.S., Ph.D., M.P.E. 5315 Drexel Ave., Chicago, Ill. *Professor of Physiology, George Williams College, Hyde Park.* (1, 1928)

Stekol, Jakob A., M.S., D.Sc. Department of Biochemistry, Vanderbilt University, Nashville, Tenn. *Assistant Professor.* (2, 1936)

Stern, Kurt G., Ph.D. 254 W. 31st St., New York City. *Research Chemist, Overly Biochemical Research Foundation.* (2, 1938)

Stevens, S. Smith, Ph.D. Emerson Hall, Harvard University, Cambridge, Mass. *Assistant Professor of Psychology.* (1, 1937)

Stewart, Colin C., Ph.D. Dartmouth College, Hanover, N. H. *Brown Professor of Physiology.* (1, 1898)

Stewart, Fred W., M.D. Memorial Hospital, 444 E. 68th St., New York City. *Pathologist; Associate Professor of Surgical Pathology, Cornell Medical School; Pathologist, New York State Department of Public Health, Division of Laboratories and Research.* (4, 1928)

Stewart, Harold L., M.D. The National Cancer Institute, Bethesda, Md. *Senior Pathologist.* (4, 1936)

Stewart, Winifred Bayard, M.D., M.A. 2028 Delancey St., Philadelphia, Pa. *Professor of Neurology, Woman's Medical College of Pennsylvania.* (1, 1941)

Stiebeling, Hazel K., M.A., Ph.D. United States Department of Agriculture, Washington, D. C. *Senior Food Economist, Bureau of Home Economics.* (5, 1933)

Stier, Theodore J. B., Ph.D. Indiana University Medical School, Bloomington. *Associate Professor of Physiology.* (1, 1938)

Still, Eugene U., Ph.D. 900 N. Franklin St., Chicago, Ill. *Director, Biochemical Research Laboratories.* (1, 1929)

Stillman, Ernest G., M.D. 45 E. 75th St., New York City. (6, 1930)

Stockton, Andrew Benton, M.D. 564 Funston Ave., San Francisco, Calif. *Assistant Clinical Professor of Medicine, Stanford Medical School.* (3, 1931)

Stokstad, E. L. Robert, Ph.D. Lederle Laboratories, Pearl River, N. Y. *Research Chemist.* (5, 1942)

Stoland, O. O., M.S., Ph.D. 1845 Learnard Ave., Lawrence, Kan. *Professor of Physiology and Pharmacology, University of Kansas.* (1, 1913)

Storey, Thomas A., Ph.D., M.D. Stanford University, Calif. *Professor of Hygiene and Physical Education and General Director of the School of Hygiene and Physical Education.* (1, 1903)

Stormont, Robert T., Ph.D. University of Chicago, Chicago, Ill. *Research Assistant in Pharmacology, Otho S. A. Sprague Memorial Institute.* (3, 1941)

Stotz, Elmer H., Ph.D. McLean Hospital, Waverley, Mass. *Research Chemist; Waverley Research Fellow, Department of Biological Chemistry, Harvard Medical School.* (2, 1939)

Stoughton, Roger W., M.S., Ph.D. Mallinckrodt Chemical Works, 3600 N. Second St., St. Louis, Mo. *Research Chemist.* (3, 1939)

Strong, Frank M., M.A., Ph.D. Department of Biochemistry, University of Wisconsin, Madison. *Associate Professor of Biochemistry.* (2, 1941)

Struck, Harold Carl, Ph.D. University of Illinois College of Medicine, 1853 W. Polk St., Chicago.

Assistant Professor of Pharmacology and Therapeutics. (1, 1940)

Stuart, Charles A., M.Sc., Ph.D. 372 Lloyd Ave., Providence, R. I. *Associate Professor of Biology, Brown University.* (6, 1935)

Sturgis, Cyrus Cressey, M.D. Simpson Memorial Institute, Ann Arbor, Mich. *Director, Thomas Henry Simpson Memorial Institute for Medical Research; Chairman, Department of Medicine, University Hospital, and Professor of Medicine, University of Michigan.* (4, 1927)

SubbaRow, Y., Ph.D. Lederle Laboratories, Pearl River, N. Y. (2, 1939)

Sugg, John Y., Ph.D. 345 E. 68th St., New York City. *Assistant Professor of Bacteriology and Immunology, Cornell Medical College.* (6, 1938)

Sullivan, Michael Xavier, Ph.D. Chemo-Medical Research Institute, Georgetown University, Washington, D. C. *Director and Research Professor of Chemistry.* (2, 1909)

Sulzberger, Marion B., M.D. 962 Park Ave., New York City. *Lieutenant Commander, M.C., U.S.N.R., in charge of Dermatology and Syphilology, U. S. Naval Hospital, Brooklyn, N. Y.; Assistant Clinical Professor of Dermatology and Syphilology, Columbia University.* (6, 1936)

Summerson, William H., M.A., Ph.D. Cornell University Medical College, 1300 York Ave., New York City. *Assistant Professor of Biochemistry.* (2, 1942)

Sumner, James Batcheller, A.M., Ph.D. Dairy Building, Ithaca, N. Y. *Professor of Biochemistry, Cornell University Medical College.* (2, 1919)

Sumwalt, Margaret, M.S., Ph.D. Medical School, University of Pennsylvania, Philadelphia. (1, 1934)

Sunderman, F. William, M.D., Ph.D. University of Pennsylvania, Philadelphia. *Assistant Professor of Research Medicine.* (2, 1931)

Sundstroem, Edward S., M.D. University of California, Berkeley. *Associate Professor of Biochemistry.* (2, 1919)

Sure, Barnett, M.S., Ph.D. University of Arkansas, Fayetteville. *Head of Department and Professor of Agricultural Chemistry.* (2, 1923; 5, 1933)

Sutherland, George F., M.D.C.M., M.Sc. Worcester State Hospital, Worcester, Mass. *Research fellow in Neuropathology, Harvard Medical School; Captain, M.C., Darnall General Hospital, Danville, Ky.* (1, 1939)

Sutton, T. Scott, M.Sc., Ph.D. Ohio State University, Columbus. *Assistant Professor; Associate, Ohio Agricultural Experiment Station, College of Agriculture.* (5, 1936)

Swain, Robert E., M.S., Ph.D., LL.D. 634 Mirada Ave., Stanford University, Calif. *Professor Emeritus of Chemistry.* (2, 1909)

Swann, Howard G., M.S., Ph.D. Dept. of Pharmacology, University of Texas Medical School, Galveston. *Assistant Professor of Physiology.* (1, 1940)

Swanson, Pearl P., M.S., Ph.D. Iowa State College, Ames. *Professor of Foods and Nutrition, Dept. of Foods and Nutrition.* (5, 1933)

Swanson, William W., M.S., M.D. 2376 E. 71st St., Chicago, Ill. (2, 1938)

Sweeney, H. Morrow, M.S., Ph.D. School of Medical Sciences, University of South Dakota, Vermillion. *Professor of Physiology and Pharmacology and Head of the Department.* (1, 1939)

Sweet, J. E., A.M., M.D., Sc.D. Unadilla, N. Y. *Emeritus Professor of Surgical Research, Cornell Medical College.* (1, 1913; 4, prior to 1920)

Swift, Homer, M.D., D.Sc. 888 Park Ave., New York City. *Member, Rockefeller Institute for Medical Research; Physician to The Hospital of The Rockefeller Institute for Medical Research.* (6, 1920)

Swift, Raymond W., M.S., Ph.D. Pennsylvania State College, State College. *Professor, Institute of Animal Nutrition.* (5, 1934)

Swingle, Wilbur Willis, Ph.D. Princeton University, Princeton, N. J. *Professor of Biology.* (1, 1924)

Sykes, Joseph F., M.S.A., Ph.D. Michigan State College, E. Lansing. *Research Assistant and Assistant Professor of Physiology and Pharmacology.* (1, 1942)

Syverton, Jerome T., M.D. The University of Rochester School of Medicine and Dentistry and Strong Memorial Hospital, Rochester, N. Y. *Associate Professor of Bacteriology.* (4, 1940)

Tainter, M. L., M.A., M.D. Stanford University School of Medicine, San Francisco, Calif. *Professor of Pharmacology.* (1, 1929; 3, 1927)

Tait, John, M.D., D.Sc., F.R.S.E., F.R.S.C. McGill University, Montreal, Que., Canada. *Professor of Physiology (Retired).* (1, 1919)

Talbert, George A., Ph.D. University Station, Grand Forks, N. D. *Professor of Physiology and Pharmacology, University of North Dakota.* (1, 1919)

Talbot, Samuel Armstrong, A.M., M.S., Ph.D. Wilmer Institute, Johns Hopkins Hospital, Baltimore, Md. *Instructor in Physiological Optics, Johns Hopkins University.* (1, 1940)

Taliaferro, William H., Ph.D. Department of Bacteriology, University of Chicago, Chicago, Ill. *Eliakim H. Moore Distinguished Service Professor of Parasitology and Dean of the Division of Biological Sciences.* (6, 1930)

Tannenbaum, Albert, M.D. Michael Reese Hospital, 29th St. & Ellis Ave., Chicago, Ill. *Director, Department of Cancer Research.* (4, 1942)

Tashiro, Shiro, Ph.D., M.D. College of Medicine, University of Cincinnati, Cincinnati, O. *Professor of Biochemistry.* (1, 1913; 2, 1913)

Tatum, Arthur L., M.S., Ph.D., M.D. University of Wisconsin, Madison. *Professor of Pharmacology.* (1, 1913; 3, 1919)

Tauber, Henry, Ph.D. Schwartz Laboratories, Inc., 202 E. 44th St., New York City. *Research Biochemist.* (2, 1933)

Taylor, Alonzo E., M.D. General Mills, Inc. 200 Chamber of Commerce, Minneapolis, Minn. *Director of Research. Director Emeritus, Food Research Institute, Stanford University.* (5, 1933)

Taylor, Fred A., Ph.D. 320 E. North Ave., N.S., Pittsburgh, Pa. *Biochemist, Singer Memorial Laboratory.* (2, 1933)

Taylor, Haywood M., M.S., Ph.D. Duke University School of Medicine, Durham, N. C. *Associate Professor of Biochemistry and Toxicology; Toxicologist to Duke Hospital.* (4, 1942)

Taylor, Norman Burke, M.D., F.R.S. (Can.), M.R.C.S. (Eng.), L.R.C.P. (Lon.), F.R.C.S. (Edin.), F.R.C.P. (Can.). University of Toronto, 5, Ontario, Ont., Canada. *Professor of Physiology.* (1, 1922)

Teague, Robert S., M.D., Ph.D. Department of Pharmacology, Tulane University of Louisiana, Station 20, New Orleans. *Instructor in Pharmacology.* (3, 1942)

Templeton, Roy D., B.S. Loyola University School of Medicine, 706 S. Lincoln St., Chicago, Ill. *Associate in Physiology.* (1, 1935)

Ten Broeck, Carl, M.D. The Rockefeller Institute for Medical Research, Department of Animal and Plant Pathology, Princeton, N. J. *Member.* (4, 1932; 6, 1924)

Terplan, Kornel L., M.D. University of Buffalo, School of Medicine, Buffalo, N. Y. *Professor of Pathology and Bacteriology.* (4, 1935)

Thannhauser, S. J., M.D., Ph.D. Tufts College Medical School, 30 Bennet St., Boston, Mass. *Clinical Professor of Medicine and Associate Physician in Chief, Joseph H. Pratt Diagnostic Hospital.* (2, 1937)

Thayer, Sidney Allen, Ph.D. 1402 S. Grand Blvd., St. Louis, Mo. *Assistant Professor of Biochemistry, St. Louis University School of Medicine.* (2, 1933)

Theiler, Max, M.D. Rockefeller Foundation, New York City. *Member of Field Staff.* (4, 1938)

Thienes, Clinton H., A.M., M.D., Ph.D. University of Southern California School of Medicine, Los Angeles. *Professor of Pharmacology.* (3, 1928)

Thomas, Arthur W., Ph.D. Columbia University, New York City. *Professor of Chemistry.* (2, 1924)

Thomas, Byron H., M.S., Ph.D. Iowa State College, Ames. *Professor and Head, Animal Chemistry and Nutrition, Iowa Agricultural Experiment Station.* (5, 1933)

Thomas, Caroline Bedell, M.D. The Johns Hopkins Hospital, Baltimore, Md. *Instructor in Medicine, Johns Hopkins University School of Medicine.* (1, 1939)

Thomas, J. Earl, M.S., M.D. Jefferson Medical College, Philadelphia, Pa. *Professor of Physiology.* (1, 1922; 3, 1924)

Thompson, Randall L., S.M., Sc.D., M.D. School of Medicine, Western Reserve University, 2109 Adelbert Rd., Cleveland, O. *Assistant Professor of Bacteriology.* (6, 1937)

Thompson, William R., Ph.D. 883 Warren St., Albany, N. Y. (2, 1934)

Thomson, David Landsborough, M.A., Ph.D. McGill University, Montreal, Canada. *Professor of Biochemistry.* (2, 1929)

Thorn, George Widmer, M.D. Peter Bent Brigham Hospital, Boston, Mass. *Professor of Medicine of Harvard University.* (1, 1939)

Tillett, William S., M.D., Sc.D. (hon.). Department of Bacteriology, New York University College of Medicine, 477 First Ave., New York City. *Professor of Medicine.* (6, 1927)

Tilt, Jennie, M.S., Ph.D. Florida State College for Women, Tallahassee. *Professor of Physiological Chemistry and Nutrition.* (5, 1937)

Tipson, R. Stuart, Ph.D. Mellon Institute of Industrial Research, University of Pittsburgh, Pittsburgh, Pa. *Fellow, Department of Research in Pure Chemistry.* (2, 1937)

Tipton, Samuel R., Ph.D. Department of Physiology, College of Medicine, Wayne University, Detroit, Mich. *Assistant Professor in Physiology.* (1, 1940)

Tisdall, Frederick F., M.S., M.D., M.R.C.S., L.R.C.P. (London), F.R.C.P. (C.). University of Toronto, Toronto, Canada. *Associate Professor of Pediatrics; Associate Physician, Hospital for Sick Children, Department of Medicine, University of Toronto.* (2, 1922; 5, 1933)

Titus, Harry W., A.M., Ph.D. 3705 24th St., N.E., Washington, D. C. *Senior Biological Chemist, Bureau of Animal Industry, U. S. Department of Agriculture.* (2, 1929; 5, 1933)

Tocantins, Leandro Maués, M.D. Jefferson Medical College, Philadelphia, Pa. *Associate Professor of Medicine.* (1, 1939)

Todhunter, Elizabeth Neige, M.Sc., Ph.D., University of Alabama, University. *Associate Professor of Nutrition.* (5, 1939)

Toennies, Gerrit, Ph.D. Lankenau Hospital Research Institute, Philadelphia, Pa. *Research Chemist.* (2, 1934)

Tolle, Chester D., Ph.D. Food and Drug Administration, Federal Security Agency, Washington, D. C. *Senior Biochemist.* (**5,** 1942)

Tompkins, Edna H., M.D. Vanderbilt University, School of Medicine, Nashville, Tenn. *Associate Professor of Anatomy.* (**4,** 1941)

Torrey, John C., Ph.D., D.Sc. 1300 York Ave., New York City. *Professor (Emeritus) of Epidemiology, Cornell University Medical College.* (**6,** 1920)

Toth, Louis A., M.S., Ph.D. Tulane University School of Medicine, Station 20, New Orleans, La. *Assistant Professor of Physiology.* (**1,** 1940)

Tourtellotte, Dee, M.S., D.Sc. Charles B. Knox Gelatin Co., Johnstown, N. Y. (**5,** 1935)

Tower, Sarah Sheldon, M.D., Ph.D. Johns Hopkins Medical School, Baltimore, Md. *Associate in Anatomy.* (**1,** 1932)

Travell, Janet, M.D. Cornell University Medical College, New York City. *Instructor in Pharmacology.* (**3,** 1933)

Travis, Lee Edward, A.M., Ph.D. University of Southern California, Los Angeles. *Professor of Psychology and Director of the Psychological Clinic.* (**1,** 1929)

Treffers, Henry P., Ph.D. Harvard Medical School, Boston, Mass. *Assistant Professor of Comparative Pathology and Biochemistry.* (**6,** 1942)

Trimble, Harry C., M.D., Ph.D. 25 Shattuck St., Boston Mass. *Assistant Professor of Biological Chemistry, Harvard Medical School.* (**2,** 1929; **5,** 1936)

Tuft, Louis H., M.D. 1530 Locust St., Philadelphia, Pa. *Assistant Professor of Medicine, Temple University Medical School; Chief of Clinic of Allergy and Applied Immunology, Temple University Hospital.* (**6,** 1928)

tum Suden, Caroline, M.A., Ph.D. 80 E. Concord St., Boston, Mass. *Evans Research Fellow in Physiology, Boston University School of Medicine; Assistant, Evans Memorial Staff, Massachusetts Memorial Hospitals.* (**1,** 1936)

Tuohy, Edward B., M.S., M.D. Mayo Foundation, Mayo Clinic, Rochester, Minn. *Assistant Professor of Anesthesiology.* (**3,** 1941)

Turner, Abby H., Ph.D. Mount Holyoke College, South Hadley, Mass. *Professor of Physiology.* (**1,** 1928)

Turner, William A., Ph.D. Bureau of Dairy Industry, U. S. Department of Agriculture, Beltsville, Md. *Associate Chemist.* (**2,** 1929)

Tuttle, Waid Wright, M.A., Ph.D. University of Iowa, Iowa City. *Professor of Physiology.* (**1,** 1925)

Tweedy, Wilbur R., Ph.D. Loyola University School of Medicine, 706 S. Wolcott St., Chicago, Ill. *Professor and Chairman, Department of Biological Chemistry.* (**2,** 1931)

Unna, Klaus R. W., M.D. Merck Institute for Therapeutic Research, Rahway, N. J. *Research Associate.* (**1,** 1941; **5,** 1942)

Upton, Morgan, M.A., Ph.D. British Purchasing Commission, 1518 K St., N.W. Washington, D. C. (**1,** 1934)

Urban, Frank, Ph.D. Washington University School of Medicine, St. Louis, Mo. *Assistant Professor of Biochemistry.* (**2,** 1932)

Vahlteich, Ella McCollum, M.A., Ph.D. 46 Hudson Ave., Edgewater, N. J. (**5,** 1933)

van Dyke, H. B., Ph.D., M.D. The Squibb Institute for Medical Research, New Brunswick, N. J. *Head of the Division of Pharmacology; Honorary Professor of Physiology, Rutgers University.* (**1,** 1925; **3,** 1942)

van Harreveld, Anthonie, M.A., M.D. California Institute of Technology, Pasadena. *Associate Professor of Physiology.* (**1,** 1941)

Van Liere, Edward J., M.S., M.D., Ph.D. The School of Medicine, West Virginia University, Morgantown. *Professor of Physiology and Dean.* (**1,** 1927)

Van Slyke, Donald D., Ph.D., Sc.D., M.D. Rockefeller Institute for Medical Research, 66th St. and York Ave., New York City. *Member; Member, National Academy of Sciences.* (**2,** 1908)

van Wagenen, Gertrude, Ph.D. Yale University School of Medicine, New Haven, Conn. *Associate Professor.* (**1,** 1932)

Van Winkle, Walton, Jr., M.D. Pharmacology Division, Food and Drug Administration, Federal Security Agency, Washington, D. C. *Associate Pharmacologist.* (**3,** 1939)

Vars, Harry M., Ph.D. Harrison Department of Surgical Research, University of Pennsylvania Medical School, Philadelphia. *Assistant Professor of Physiological Chemistry.* (**2,** 1935; **5,** 1935)

Venning, Eleanor H., M.S., Ph.D. University Clinic, Royal Victoria Hospital, Pine Ave., Montreal, Quebec, Canada. *Research Fellow.* (**2,** 1938)

Vickery, Hubert B., M.S., Ph.D. Connecticut Agricultural Experiment Station, New Haven. *Lecturer on the Chemistry of Proteins, Yale University; Biochemist in Charge, Connecticut Agricultural Experiment Station.* (**2,** 1923)

Victor, Joseph, M.D. Research Division for Chronic Disease, Welfare Island, N. Y. *Experimental Pathologist, Associate in Pathology, Columbia University College of Physicians and Surgeons.* (**4,** 1935)

Virtue, Robert W., Ph.D. University of Denver, Denver, Colo. *Associate Professor of Chemistry.* (**2,** 1939)

Visscher, Maurice B., M.S., Ph.D. University of Minnesota, Minneapolis. *Professor of Physiology.* (1, 1927)

Voegtlin, Carl, Ph.D. National Cancer Institute, Bethesda, Md. *Pharmacologist Director, United States Public Health Service.* (1, 1908; 2, 1908; 3, 1908)

von Haam, Emmerich, M.D. Ohio State University, Columbus. *Professor of Pathology.* (4, 1938)

Von Oettingen, W. F., M.D., Ph.D. National Institute of Health, Division of Industrial Hygiene, Bethesda, Md. *Principal Industrial Toxicologist.* (3, 1925)

Vorwald, Arthur J., Ph.D., M.D. Lt. Commander, U.S.N.R., U. S. Naval Hospital, Annapolis, Md. (4, 1937)

Vos, Bert J., Ph.D., M.D. Food and Drug Administration, Washington, D. C. *Associate Pharmacologist.* (3, 1941)

Waddell, J. A., M.D. Monroe Hill, Medical School, University of Virginia, Charlottesville. *Professor of Pharmacology.* (3, 1916)

Waddell, James, Ph.D. E. I. duPont de Nemours & Co., New Brunswick, N. J. *Director of the Biological Laboratory.* (2, 1930; 5, 1935)

Wadsworth, Augustus B., M.D. New York State Department of Health, Albany. *Director, Division of Laboratories and Research.* (4, 1935; 6, 1920)

Waelsch, Heinrich, M.D., Ph.D. Columbia University, College of Physicians and Surgeons, 630 W. 168th St., New York City. *Research Associate in Biochemistry.* (2, 1941)

Wakeman, Alfred J., Ph.D. Hatfield Hill Road, Bethany, Conn. *Retired.* (2, 1906)

Wakerlin, George E., Ph.D., M.D. University of Illinois Medical School, 1853 W. Polk St., Chicago. *Professor of Physiology.* (1, 1933; 3, 1934)

Wakim, Khalil G., M.D., Ph.D. University of Indiana Medical School, Bloomington. *Associate Professor of Physiology.* (1, 1942)

Wald, George, M.A., Ph.D. Biological Laboratories, Harvard University, Cambridge, Mass. (1, 1934)

Walker, Arthur M., M.D. University of Pennsylvania, Philadelphia. *Associate Professor of Pharmacology.* (1, 1932; 3, 1939)

Walker, Burnham S., Ph.D., M.D. Boston University School of Medicine, 80 E. Concord St., Boston, Mass. *Professor of Biochemistry.* (2, 1940)

Walker, Ernest Linwood, S.D. Second and Parnassus Aves., San Francisco, Calif. *Professor of Tropical Med., The George Williams Hooper Foundation for Medical Research, University of California.* (3, 1931)

Wallace, Edward W., Ph.D., M.D. 1203 Ryland Ave., Cincinnati, O. *Associate Professor of Pharmacology, University of Cincinnati, College of Medicine.* (3, 1938)

Wallace, George B., M.D. 477 First Ave., New York City. *Professor of Pharmacology, New York University College of Medicine.* (1, 1901; 2, 1906; 3, 1909)

Wallen-Lawrence, Zonja, Ph.D. 4534 W. Pine Blvd., St. Louis, Mo. (2, 1937)

Walter, Carl W., M.D. Harvard Medical School, 25 Shattuck Street, Boston, Mass. *Director, Laboratory for Surgical Research; Associate in Surgery, Peter Bent Brigham Hospital.* (4, 1942)

Walters, Orville S., Ph.D., M.D. Central College, McPherson, Kan. *President.* (1, 1936)

Walton, Robert P., M.A., Ph.D., M.D. Medical College of the State of South Carolina, Charleston. *Professor of Pharmacology.* (3, 1933)

Walton, Seth T., V.M.D., M.S., Ph.D. City Health Department, Charlotte, N. C. *Director of Laboratories and Research.* (6, 1936)

Walzer, Matthew, M.D. 20 Plaza St., Brooklyn, N. Y. *Attending in Allergy, Jewish Hospital of Brooklyn.* (6, 1924)

Wang, Chi Che, M.S., Ph.D. 323 Belden Ave., Lincoln Park Sta., Chicago, Ill. *Research Chemist, Northwestern Yeast Co.* (2, 1922; 5, 1933)

Wangensteen, Owen Harding, M.D. University of Minnesota, Minneapolis. *Professor of Surgery.* (4, 1931)

Warner, Emory D., M.D. Medical Laboratories Bldg., Iowa City, Ia. *Associate Professor of Pathology.* (4, 1937)

Warren, Charles O., Ph.D., M.D. Cornell University Medical College, 1300 York Ave., New York City. *Assistant Professor of Physiology and Anatomy.* (1, 1941)

Warren, Madeleine Field, A.M., Ph.D. Harvard School of Public Health, 55 Shattuck St., Boston, Mass. *Associate in Physiology.* (1, 1933)

Warren, Shields, M.D. Palmer Memorial Hospital, 195 Pilgrim Rd., Boston, Mass. *Pathologist, New England Deaconess Hospital; Assistant Professor of Pathology, Harvard Medical School.* (4, 1929)

Wartman, William Beckmann, M.D. Western Reserve University, 2085 Adelbert Rd., Cleveland, O. *Assistant Professor of Pathology.* (4, 1940)

Wasteneys, Hardolph, Ph.D., F.R.S.C. University of Toronto, Toronto, Canada. *Professor and Head of Department of Biochemistry.* (2, 1915)

Wastl, Helene, M.D. Hahnemann Medical College and Hospital, Philadelphia, Pa. *Research*

Associate in Pharmacology and Anatomy. (1, 1939)

Waterman, Robert E., B.S. Research Corporation, 405 Lexington Ave., New York City. (2, 1940)

Waters, Ralph Milton, M.D. University of Wisconsin, Madison. *Professor of Anesthesia.* (3, 1937)

Watson, Cecil J., M.D., Ph.D. Department of Medicine, University Hospital, Minneapolis, Minn. *Professor of Medicine.* (4, 1941)

Watson, John B., A.M., Ph.D., LL.D. 420 Lexington Ave., New York City. *Vice President of the J. Walter Thompson Co.* (1, 1907)

Waud, Russell A., M.D., M.Sc., Ph.D. Medical School, University of Western Ontario, London, Canada. *Professor of Pharmacology.* (1, 1925; 3, 1931)

Wearn, Joseph T., M.D. Lakeside Hospital, Cleveland, O. *Professor of Medicine, Western Reserve University; Director of Medicine, Lakeside Hospital.* (1, 1921)

Weatherby, J. H., M.A., Ph.D. Medical College of Virginia, Richmond. *Research Associate in Pharmacology.* (3, 1941)

Weber, Clarence J., M.D., Ph.D. University of Kansas Hospitals, Kansas City. *Assistant Professor of Research Medicine.* (2, 1931)

Webster, Bruce, M.D., C.M. Cornell University Medical College, 1300 York Ave., New York City. *Assistant Professor Medicine; Associate Attending Physician, New York Hospital.* (5 1935)

Webster, Leslie T., M.D. Rockefeller Institute for Medical Research, 66th St. and York Ave., New York City. *Member, Division of Pathology and Bacteriology.* (4, 1927)

Weed, Lewis H., A.M., M.D., Sc.D. Johns Hopkins University Medical School, Baltimore, Md. *Professor of Anatomy.* (1, 1919)

Wégria, René, M.D. City Hospital, Scranton Road, Cleveland, O. *Junior Assistant Resident in Medicine.* (1, 1941)

Weichert, Charles K., Ph.D. University of Cincinnati, Cincinnati, O. *Assistant Professor of Zoology.* (1, 1935)

Weil, Alfred J., M.D. Lederle Laboratories, Inc., Pearl River, N. Y. *Immunologist.* (6, 1940)

Weil, Arthur, M.D. Northwestern University Medical School, 303 E. Chicago Ave., Chicago, Ill. *Associate Professor of Neuropathology.* (4, 1940)

Weil, Leopold, Ph.D. Eastern Regional Research Laboratory, U. S. Department of Agriculture, Chestnut Hill Station, Philadelphia, Pa. *Associate Chemist.* (2, 1942)

Weir, Everett G., M.S., Ph.D. School of Medicine, Howard University, Washington, D. C. *Assistant Professor of Physiology.* (1, 1941)

Weiss, Charles, M.S., Ph.D., M.D. Mount Zion Hospital, San Francisco, Calif. *Associate Professor of Research Medicine and Lecturer in Pediatrics, University of California; Director of Clinical and Research Laboratories, Mount Zion Hospital.* (4, 1934; 6, 1920)

Weiss, Emil, M.D., Ph.D. 2318 Irving Park Rd., Chicago, Ill. *Pathologist, Chicago Eye, Ear, Nose and Throat Hospital.* (6, 1927)

Weiss, Paul, Ph.D. University of Chicago, Chicago, Ill. *Professor of Zoology.* (1, 1936)

Welch, Arnold DeMerritt, Ph.D., M.D. Medical Research Division, Sharp and Dohme, Inc., Glenolden, Pa. *Director of Pharmacological Research.* (3, 1942)

Welch, Henry, Ph.D. Bacteriological Section, U. S. Food and Drug Administration, Washington, D. C. *Senior Bacteriologist.* (6, 1932)

Weld, Charles Beecher, M.A., M.D. Dalhousie University, Halifax, N.S., Canada. *Professor of Physiology.* (1, 1936)

Weld, Mrs. Julia T. College of Physicians and Surgeons, 630 W. 168th St., New York City. *Research Associate in Pathology.* (6, 1920)

Welker, William H., A.C., Ph.D., D.Sc. 1853 W. Polk St., Chicago, Ill. *Professor of Physiological Chemistry and Head of the Department, College of Medicine, University of Illinois.* (2, 1906)

Weller, Carl Vernon, M.D. 1130 Fair Oaks Parkway, Ann Arbor, Mich. *Professor of Pathology and Chairman, Department of Pathology, University of Michigan.* (4, 1923)

Wells, H. Gideon, M.D. Department of Pathology, University of Chicago, Chicago, Ill. *Professor Emeritus; Director Emeritus of the Otho S. A. Sprague Memorial Institute; Member National Academy of Sciences.* (2, 1906; 4, prior to 1920)

Wells, Herbert S., M.D. Bowman Gray School of Medicine, Winston-Salem, N. C. *Professor of Physiology and Pharmacology.* (1, 1932)

Wendel, William B., Ph.D. College of Medicine, University of Tennessee, Memphis. *Associate Professor of Chemistry.* (2, 1932)

Werkman, C. H., Ph.D. Science Hall, Iowa State College, Ames. *Professor in Charge, Bacteriology Section.* (2, 1942)

Werner, Harold W., Ph.D. Division of Industrial Hygiene and Toxicology, National Institute of Health, Bethesda, Md. *Pharmacologist.* (3, 1942)

Wesson, Laurence Goddard, Ph.D. Forsyth Dental Infirmary, Boston, Mass. *Research Biochemist.* (2, 1922; 3, 1932)

West, Edward S., M.S., Ph.D. University of Oregon Medical School, Portland. *Professor of Biochemistry.* (2, 1925)

West, Randolph, M.A., M.D. 622 W. 168th St., New York City. *Associate Professor of Medicine, Columbia University.* (2, 1931)

Weymouth, Frank W., Ph.D. Stanford University, Calif. *Professor of Physiology and Executive of the Department.* (1, 1917)

Wheeler, George W., M.D. New York Hospital, 525 E. 68th St., New York City. *Superintendent.* (6, 1920)

Wheeler, Kenneth M., Ph.D. Bureau of Laboratories, Connecticut State Department of Health, 1179 Main St., Hartford. *Research Microbiologist.* (6, 1938)

Wheeler, Mary W., M.A. Division of Laboratories and Research, New York State Department of Health, Albany. *Associate Bacteriologist.* (6, 1933)

Wheeler, Ruth, Ph.D. Vassar College, Poughkeepsie, N. Y. *Professor of Physiology and Nutrition.* (2, 1915; 5, 1933)

Wheelon, Homer, M.S., M.D. American Bank Bldg., Seattle, Wash. (1, 1919)

Whipple, George H., M.D., Sc.D. University of Rochester, Rochester, N. Y. *Professor of Pathology and Dean of the School of Medicine and Dentistry; Member of the National Academy of Sciences.* (1, 1911; 4, prior to 1920)

White, Abraham, M.A., Ph.D. 333 Cedar St., New Haven, Conn. *Assistant Professor of Physiological Chemistry, Medical School, Yale University.* (2, 1934; 5, 1937)

White, Frank D., Ph.D., F.I.C. Medical College, University of Manitoba, Winnipeg, Canada. *Assistant Professor of Biochemistry, Faculty of Medicine.* (2, 1931)

White, Harvey Lester, M.D. Washington University Medical School, St. Louis, Mo. *Associate Professor of Physiology on Leave; Lt. Col. M.C.* (1, 1923)

White, Julius, A.M., Ph.D. National Cancer Institute, Bethesda, Md. *Senior Biochemist.* (2, 1937)

White, Paul Dudley, M.D., Massachusetts General Hospital, Boston. *Lecturer in Medicine, Harvard Medical School; Physician (in charge of Cardiac Clinics and Laboratory), Mass. General Hospital.* (3, 1921)

Whitehead, Richard W., M.A., M.D. University of Colorado School of Medicine, 4200 E. Ninth Ave., Denver. *Professor of Physiology and Pharmacology.* (1, 1933; 3, 1928)

Wiener, Alexander S., M.D. 64 Rutland Rd., Brooklyn, N. Y. *Bacteriologist and Serologist to Office of Chief Medical Examiner of New York City; Head of Transfusion Division, Jewish Hospital of Brooklyn.* (6, 1932)

Wiersma, Cornelis A. G., M.A., Ph.D. California Institute of Technology, Pasadena. *Associate Professor of Physiology.* (1, 1941)

Wiggers, Carl J., M.D., Sc.D. Medical School, Western Reserve University, Cleveland, O. *Professor and Director of Physiology.* (1, 1907; 3, 1909)

Wiggers, Harold C., Ph.D. Western Reserve University Medical School, 2109 Adelbert Rd., Cleveland, O. *Assistant Professor of Physiology.* (1, 1938)

Wilder, Russell M., Ph.D., M.D. Mayo Clinic, Rochester, Minn. *Professor of Medicine, Mayo Foundation, University of Minnesota.* (1, 1921; 4, 1924; 5, 1933)

Wiley, Frank H., M.S., Ph.D. Food and Drug Administration, Federal Security Agency, Washington, D. C. *Senior Chemist.* (2, 1933)

Wilhelmi, Alfred E., Ph.D. 333 Cedar St., New Haven, Conn. Yale University School of Medicine. *Instructor in Physiological Chemistry.* (2, 1942)

Wilhelmj, Charles Martel, M.D. Creighton University School of Medicine, Omaha, Neb. *Professor of Physiology.* (1, 1931)

Wilkerson, Vernon A., M.D., Ph.D. Howard University Medical School, Washington, D. C. *Professor and Head of Department of Biochemistry.* (2, 1936)

Williams, Harold H., Ph.D. 660 Frederick St., Detroit, Mich. *Associate Director, Research Laboratory, Children's Fund of Michigan.* (2, 1938; 5, 1936)

Williams, Horatio B., M.D., Sc.D. 632 W. 168th St., New York City. *Dalton Professor of Physiology, Columbia University.* (1, 1912)

Williams, Ray D., M.D. 6372 Alamo Ave., St. Louis, Mo. *Research Fellow.* (5, 1941)

Williams, Robert R., D.Sc. Bell Telephone Laboratories, 297 Summit Ave., Summit, N. J. *Chemical Director.* (5, 1941)

Williams, Robert Hardin, M.D. Thorndike Laboratory, Boston City Hospital, Boston, Mass. *Instructor in Medicine, Harvard Medical School; Assistant Physician, Thorndike Memorial Laboratory; Junior Visiting Physician, II and IV Medical Services (Harvard) Boston City Hospital.* (4, 1940)

Williams, Robert R., M.S., D.Sc. 297 Summit Ave., Summit, N. J. *Chemical Director, Bell Telephone Laboratories.* (2, 1919)

Williams, Roger J., Ph.D., D.Sc. University of Texas, Department of Chemistry, Austin. *Professor of Chemistry.* (2, 1931)

Wilson, David Wright, M.S., Ph.D. University of Pennsylvania Medical School, Philadelphia. *Professor of Physiological Chemistry.* (1, 1915; 2, 1915)

Wilson, Frank N., M.D. University Hospital, Ann Arbor, Mich. *Professor of Medicine, University of Michigan.* (4, 1925)

Wilson, Karl M., M.D. University of Rochester, School of Medicine, Rochester, N. Y. *Professor of Obstetrics and Gynecology.* (4, 1927)

Wilson, P. W., Ph.D. Department of Agricultural Bacteriology, University of Wisconsin, Madison. *Associate Professor in Agricultural Bacteriology.* (2, 1939)

Wilson, Robert H., Ph.D. U. S. Department of Agriculture, Stanford University School of Medicine, San Francisco, Calif. *Pharmacologist.* (3, 1937)

Winder, Claude V., Sc.D. Medical School, University of Michigan, Ann Arbor. *Assistant Professor of Physiology.* (1, 1938)

Windle, William Frederick, Ph.D. Northwestern University Medical School, 303 E. Chicago Ave., Chicago, Ill. *Professor of Anatomy.* (1, 1937)

Winkenwerder, Walter LaF., M.D. 1014 St. Paul St., Baltimore, Md. *Associate in Medicine, Johns Hopkins Medical School.* (6, 1938)

Winkler, Alexander Woodward, A.M., M.D. New Haven Hospital, 789 Howard Ave., New Haven, Conn. *Assistant Professor of Medicine, Yale University School of Medicine.* (1, 1940)

Winter, Charles A., Ph.D. State University of Iowa, College of Medicine, Iowa City. *Associate in Physiology.* (1, 1940)

Winter, Irwin Clinton, Ph.D., M.D. University of Oklahoma School of Medicine, Oklahoma City. *Associate Professor in Pharmacology.* (3, 1941)

Winters, Jet C., M.A., Ph.D. University of Texas, Austin. *Professor of Home Economics.* (5, 1933)

Winternitz, M. C., M.D. Yale University School of Medicine, New Haven, Conn. *Anthony N. Brady Professor of Pathology.* (4, prior to 1920)

Wintersteiner, Oskar, Ph.D. The Squibb Institute for Medical Research, New Brunswick, N. J. *Head, Division of Organic Chemistry; Honorary Professor of Biochemistry, Rutgers University.* (2, 1930)

Wintrobe, Maxwell Myer, M.D., Ph.D. The Johns Hopkins Hospital, Baltimore, Md. *Associate in Medicine, Johns Hopkins University; Associate Physician, Johns Hopkins Hospital; and Physician in Charge of Clinic for Nutritional, Gastro-intestinal and Hemopoietic Disorders. Editor, Bull. Johns Hopkins Hospital.* (4, 1940)

Wiseman, Bruce Kenneth, M.D. Kinsman Hall, Ohio State University, Columbus. *Professor of Medicine; Assistant Director of Medical Research.* (4, 1932)

Wislocki, George B., M.D. Harvard University Medical School, 25 Shattuck St., Boston, Mass. *Parkman Professor of Anatomy.* (1, 1924)

Witebsky, Ernest, M.D. Buffalo General Hospital, 100 High St., Buffalo, N. Y. *Professor of Bacteriology and Immunology.* (6, 1935)

Witzemann, Edgar J., M.A., Ph.D. Service Memorial Building, University of Wisconsin, Madison. *Associate Professor of Physiological Chemistry.* (2, 1925)

Wolbach, S. Burt, M.D. Harvard University Medical School, 25 Shattuck St., Boston, Mass. *Shattuck Professor of Pathological Anatomy; Member, National Academy of Sciences.* (4, prior to 1920)

Wolff, Harold G., M.D., M.A. New York Hospital, 525 E. 68th St., New York City. *Associate Professor of Medicine, Cornell University Medical College; Associate Attending Physician, New York Hospital.* (1, 1930; 3, 1942)

Wood, Horatio C., Jr., M.D., Ph.M. 319 S. 41st St., Philadelphia, Pa. *Professor of Pharmacology and Therapeutics, University of Pennsylvania; Professor of Materia Medica, Philadelphia College of Pharmacy and Science.* (3, 1908)

Woodbury, Robert A., Ph.D., M.D. University of Georgia, School of Medicine, Augusta. *Professor of Pharmacology.* (1, 1936; 3, 1941)

Woodruff, Lorande Loss, A.M., Ph.D. Yale University, New Haven, Conn. *Professor of Protozoology; Member, National Academy of Sciences.* (1, 1910)

Woods, Alan C., M.D. Wilmer Institute, Johns Hopkins Hospital, Baltimore, Md. *Ophthalmologist-in-Chief; Acting Professor of Ophthalmology, Johns Hopkins University; Director, Wilmer Ophthalmological Institute.* (6, 1918)

Woods, Ella, A.M., Ph.D. University of Idaho, Moscow. *Home Economist, Experiment Station.* (2, 1925; 5, 1933)

Woodward, Alvalyn E., M.S., Ph.D. University of Michigan, Ann Arbor. *Assistant Professor of Zoology.* (1, 1932)

Woodyatt, Rollin T., M.D. 237 E. Deleware Place, Chicago, Ill. *Professor of Medicine, Rush Medical College, University of Chicago.* (2, 1912)

Woolley, Dillworth W., Ph.D. Rockefeller Institute for Medical Research, 66th St., and York Ave., New York City. *Fellow.* (5, 1941)

Woolsey, Clinton N., M.D. Johns Hopkins University School of Medicine, Baltimore, Md. *Fellow in Orthopedic Surgery.* (1, 1938)

Wright, Angus, M.D. University of Southern California Medical School, 657 S. Westlake Ave., Los Angeles. *Instructor in Pathology; Pathologist, California Hospital.* (4, 1935)

Wright, Arthur W., M.D. Albany Medical College, New Scotland Ave., Albany, N. Y. *Professor of Pathology and Bacteriology.* (4, 1941)

Wright, Charles Ingham, M.S., Ph.D. National Institute of Health, Bethesda, Md. *Pharmacologist, U. S. Public Health Service.* (1, 1935; 3, 1936)

Wright, Harold N., M.S., Ph.D. University of Minnesota, Minneapolis. *Associate Professor of Pharmacology.* (3, 1933)

Wright, Sydney L., M.A., Ph.D. Endsmeet Farm, Glenside, Pa. (2, 1933)

Wulzen, Rosalind, M.S., Ph.D. Oregon State College, Corvallis. *Assistant Professor of Zoology.* (1, 1916)

Wyckoff, Ralph W. G., Ph.D. Reichel Laboratories, Kimberton, Pa. *Technical Director.* (6, 1940)

Wyman, Jeffries, Jr., Ph.D. Harvard University, Cambridge, Mass. *Associate Professor of Zoology and Chairman of the Board of Tutors in Division of Biology.* (1, 1928)

Wyman, Leland C., Ph.D. Boston University School of Medicine, Boston, Mass. *Associate Professor of Physiology.* (1, 1927)

Wynne, Arthur M., Ph.D. University of Toronto, Toronto, Canada. *Professor of Biochemistry.* (2, 1940)

Yagle, Elizabeth M., Ph.D. 2808 W. Grand Blvd., Detroit, Mich. *Serologist, Henry Ford Hospital.* (6, 1930)

Yerkes, Robert M., Ph.D. Yale Laboratories of Primate Biology, 333 Cedar St., New Haven, Conn. *Professor of Psychobiology, Yale University; Member of the National Academy of Sciences.* (1, 1904)

Yonkman, Frederick F., Ph.D., M.D. Wayne University College of Medicine, Detroit, Mich. *Professor of Pharmacology and Therapeutics.* (3, 1931)

Youmans, William Barton, M.A., Ph.D. University of Oregon Medical School, Portland. *Assoc. Professor of Physiology.* (1, 1939)

Young, A. G., Ph.D., M.D. 520 Commonwealth Ave., Boston, Mass. *Assistant Professor of Therapeutics, Boston University School of Medicine; Medical Director, Corey Hill Hospital, Brookline.* (3, 1925)

Young, E. G., Ph.D., F.R.S.C. Dalhousie University, Halifax, N. S., Canada. *Professor of Biochemistry.* (2, 1925)

Youngburg, Guy E., M.S., Ph.D. 179 Admiral Rd., Buffalo, N. Y. *Professor of Biological Chemistry, University of Buffalo.* (2, 1927)

Yuile, Charles L., M.D., C.M. Pathological Institute, McGill University, Montreal, Canada. *Lecturer in Pathology.* (4, 1941)

Zechmeister, L. California Institute of Technology, Pasadena. *Professor of Organic Chemistry.* (2, 1941)

Zeckwer, Isolde T., M.D. School of Medicine, University of Pennsylvania, Philadelphia. *Assistant Professor of Pathology.* (1, 1934; 4, 1927)

Zimmerman, Harry M., M.D. Yale University School of Medicine, 310 Cedar St., New Haven, Conn. *Associate Professor of Pathology.* (4, 1933)

Zwemer, Raymund L., Ph.D. College of Physicians and Surgeons, Columbia University, 630 W. 168th St., New York City. *Assistant Professor of Anatomy.* (1, 1930)

SUMMARY OF MEMBERSHIP

The American Physiological Society	796
The American Society of Biological Chemists	612
The American Society for Pharmacology and Experimental Therapeutics	285
The American Society for Experimental Pathology	302
The American Institute of Nutrition	266
American Association of Immunologists	242
Total Members by Societies	2503

DECEASED MEMBERS

Abel, John J. (1, 2, 3) May 26, 1938.
Abbott, A. C. (1) September 11, 1936.
Abramson, H. L. (6) April, 1934.
Adami, J. George (2) August 29, 1926.
Adler, Herman M. (2) December 6, 1935.
Adler, Isaac (3) February 2, 1912.
Alsberg, Carl L. (1, 2) October 31, 1940.
Armsby, H. P. (1) October 19, 1921.
Atkinson, Harry V. (3) May 7, 1939.
Atwater, W. O. (1) September 22, 1907.
Austin, William C. (2) November 20, 1935.
Bancroft, F. W. (1) August 23, 1924.
Banting, F. G. (3) February 21, 1941.
Banzhaf, Edwin J. (2, 6) March 17, 1931.
Benedict, Stanley R. (1, 2) December 21, 1936.
Beyer, Henry G. (1) December 9, 1918.
Black, Otis Fisher (2) October 14, 1933.
Blackfan, Kenneth D. (5) November 5, 1941.
Bleile, Albert M. (1) August 16, 1933.
Bodansky, Meyer (2) June 14, 1941.
Bowditch, Henry P. (1) March 13, 1911.
Braman, Winfred W. (5) March 24, 1937.
Brodie, Maurice (6) May 9, 1939.

Brodie, Thomas G. (1, 2) August 20, 1916.
Brown, Wade H. (3, 4) August 4, 1942.
Bull, Carroll G. (6) May 30, 1931.
Burget, G. E. (1) June 4, 1938.
Busch, Fred C. (1) January 3, 1914.
Callison, William E. (3) February 26, 1937.
Chapman, Henry C. (1) September 7, 1909.
Chillingworth, F. P. (1) June 30, 1938.
Clark, Admont Halsey (1) October 13, 1918.
Clark, G. P. (1) September 1, 1907.
Cleghorn, Allen M. (1) March 20, 1916.
Cohen, Seymour J. (3) June 11, 1942.
Connor, Charles L. (4) June 12, 1941.
Cook, Frank C. (2) June 19, 1923.
Coulter, Calvin B. (4) May 10, 1940.
Crawford, Albert C. (3) March 14, 1921.
Cullen, Glenn E. (2, 5) April 11, 1940.
Curtis, John G. (1) September 20, 1913.
Cushing, Harvey (1, 4) October 7, 1939.
Cushny, A. R. (1) February 25, 1926.
Dalton, J. C. (1) February 12, 1889.
Dastré, A. (1h) October 25, 1917.
D'Aunoy, Joseph Rigney (4) September 17, 1941.
Davis, Alice Rohde (2) August 22, 1933.
Dawson, Wilfred T. (1, 3) September 19, 1939.
Denis, Willey (1, 3) January 9, 1929.
Donaldson, Henry H. (1) January 24, 1938.
Dooley, David H. (1) April 11, 1927.
Dreyer, George P. (1) February 27, 1931.
Dunham, Edward K. (2) April 16, 1922.
Dusser de Barenne, J. G. (1, 3) June 9, 1940.
Edmunds, Charles W. (1, 3) March 1, 1941.
Englemann, Th. W. (1h) May 20, 1909.
Ewing, Ephraim MacDonald (1) August 27, 1925.
Fitch, Richard H. (1, 3) January 7, 1939.
Fitz, George W. (1) October 28, 1934.
Folin, Otto (1, 2, 3) October 26, 1934.
Foster, Nellis Barnes (2) August 20, 1933.
Franz, Shepherd Ivory (1) October 14, 1933.
Gates, Frederick L. (3, 4) June 17, 1933.
Gay, Frederick P. (4, 6) July 14, 1939.
Goodale, George L. (1) April 12, 1923.
Gortner, Ross A. (2) September 30, 1942.
Greeley, A. W. (1) May 15, 1904.
Gross, Louis (4) October 17, 1937.
Hall, G. Stanley (1) April 24, 1924.
Halsted, William S. (4) September 7, 1922.
Hammersten, O. (1h) September 21, 1932.
Harding, Victor John (2) July 10, 1934.
Hare, Hobart Amory (1) June 15, 1931.
Haskins, Howard Davis (1, 2) November 19, 1933.
Hawkins, James A., (1, 2) July 26, 1937.
Henderson, Lawrence J. (1, 2) February 10, 1942.
Herter, C. H. (1) December 5, 1910.
Hess, Alfred Fabian (2, 5) December 5, 1933.
Hewlett, Albion Walter (1, 3, 4) November 10, 1925.
Hirschfelder, Arthur D. (1, 2, 3) October 11, 1942.
Hiss, Philip H., Jr. (2, 3) February 27, 1913.
Hofmeister, F. (1h) July 26, 1922.
Hooper, Charles Warren (1) January 27, 1936.
Hough, Theodore (1) November 30, 1924.
Howland, John (2) June 20, 1926.
Huber, G. Carl (1) December 26, 1934.
Jackson, Holmes C. (1, 2) October 25, 1927.
Jaffe, Hermann R. (4, 6) December 17, 1937.
James, Wm. (1) August 26, 1910.
Jenkins, Oliver P. (1) January 9, 1935.
Jones, Frederic S. (4) October 19, 1934.
Jones, Walter (1, 2) February 28, 1935.
Jordan, Edwin O. (1) September 2, 1936.
Joseph, Don R. (1, 3) July 9, 1928.
Kahn, Max (2) April 8, 1926.
Kastle, Joseph H. (1, 2) September 24, 1916.
King, Walter E. (6) May 1, 1936.
Klotz, Oskar (4) November 3, 1936.
Koch, Waldemar (3) February 2, 1912.
Koessler, Karl K. (2, 4, 6) February 13, 1928.
Kriss, Max (5) November 15, 1941.
Krumwiede, Charles (6) December 29, 1930.
Langley, J. N. (1) November 5, 1925.
Langworthy, Charles F. (2) March 3, 1932.
Lee, Frederic S. (1) December 14, 1939.
Leech, Paul Nicholas (3) January 14, 1941.
Levene, Phoebus A. (1, 2) September 6, 1940.
Lewis, Dean (1) October 9, 1941.
Lewis, Paul A. (3, 4, 6) June 30, 1929.
Lingle, D. J. (1) November 27, 1936.
Loeb, Jacques (1, 2) February 11, 1924.
Loevenhart, A. S. (1, 2, 3) April 20, 1929.
Long, John H. (2) June 14, 1918.
Lombard, Warren P. (1) July 13, 1939.
Lusk, Graham (1, 2, 5) July 18, 1932.
Lyon, Elias P. (1) May 4, 1937.
Macallum, Archibald Byron (1, 2) April 5, 1934.
Macleod, John James Rickard (1) March 16, 1935.
McCordock, Howard A. (4) November 13, 1938.
McGlone, Bartgis (1) November 10, 1941.
McKinley, Earl B. (4, 6) July 28, 1938.
Magnus, Rudolf (3) July 25, 1927.
Mall, Franklin P. (1) November 17, 1917.
Mallory, F. B. (4) September 28, 1941.
Mandel, John A. (1, 2) May 5, 1929.
Mann, Gustav (1) July 18, 1921.
Marriott, W. McKim (2, 5) November 11, 1936.
Marshall, John (1, 2) January 5, 1925.
Martin, Ernest Gale (1) October 17, 1934.
Martin, H. Newell (1) October 27, 1896.
Matson, Ray W. (6) September, 1934.
Mathews, Samuel A. (1, 3) February 19, 1928.
Maximow, Alexander A. (4) December 4, 1928.
Maxwell, S. S. (1) January 28, 1939.
Meigs, Edward B. (1, 2, 5) November 5, 1940.
Mellus, E. Linden (1) December 17, 1923.
Meltzer, S. J. (1, 2, 3, 4) November 7, 1920.
Mendel, Lafayette B. (1, 2, 3, 5) December 9, 1935.
Meyer, Hans H. (3h) October 6, 1939.
Miller, Elmer S. (2) June 11, 1941.
Miller, Joseph L. (1, 3) August 6, 1937.

Mills, Thomas W. (1) February 13, 1915.
Minot, Charles S. (1) November 19, 1914.
Mitchell, S. Weir (1) January 4, 1914.
Moore, Lillian Mary (1) August 1, 1929.
Morris, J. Lucien (2) March 19, 1926.
Moyer, Laurence S. (2) June 8, 1942.
Myers, Harold B. (3) March 16, 1937.
Neuhausen, Benj. S. (2) August 20, 1923.
Nelson, Louis (3) April 14, 1912.
Nichols, Henry J. (4) September 2, 1927.
Noguchi, Hideyo (4, 6) May 21, 1928.
Osborne, Thomas Burr (1, 2) January 29, 1929.
Osler, Sir William (1) December 29, 1919.
Ott, Isaac (1, 3) January 1, 1916.
Park, William H. (4, 6h) April 6, 1939.
Pavlov, Ivan P. (1h) February 27, 1936.
Pearce, Richard M., Jr. (4) February 16, 1930.
Perla, David (4, 6) June 14, 1940.
Peters, H. C. (1) July 13, 1942.
Pettibone, C. J. V. (2) March 8, 1929.
Pfaff, Franz (1, 2) September 26, 1926.
Pflüger, E. (1h) March 17, 1910.
Pincussen, Ludwig (2) November 30, 1941.
Plant, Oscar H. (1, 3) October 1, 1939.
Prince, Alexander L. (1) May 25, 1938.
Ranson, S. W. (1) August 30, 1942.
Reichert, Edward T. (1) December 25, 1931.
Richards, Herbert M. (2) January 9, 1928.
Robertson, T. Brailsford (2) January 27, 1930.
Rockwood, Elbert W. (2) July 17, 1935.
Rosenbloom, Jacob (2) September 25, 1923.
Rose, Mary Schwartz (1, 2, 5) February 1, 1941.
Ross, Ellison, L. (2, 3) December 21, 1938.
Rowe, Allan Winter (1, 2, 5) December 6, 1934.
Rutan, Robert F. (2) February 19, 1930.
Schafer, Sir Edward Sharpey (1h) March 29, 1935.
Schiff, Fritz (6) 1940.
Schoenheimer, Rudolf (2) September 11, 1941.
Scott, J. M. Duncan (1) January 28, 1930.
Sedgwick, William T. (1) January 26, 1921.
Sewall, Henry (1) July 8, 1936.
Shaw, Louis A. (1) August 27, 1940.
Sheldon, Ralph E. (1) July 9, 1918.
Shorey, Edmund C. (2) January 30, 1939.
Simon, Charles E. (1, 2) November 8, 1927.
Sinclair, A. N. (6) October 21, 1930.
Simpson, G. E. (2) December 23, 1927.
Simpson, Sutherland (1) March 2, 1926.
Smith, H. E. (1) October 9, 1933.
Smith, R. Meade (1) 1919.
Smith, Theobald (4h, 6) December 10, 1934.
Spaeth, Reynold A. (1) January 26, 1925.
Sternberg, G. M. (1) November 3, 1915.
Stevens, Herman C. (1) May 27, 1934.
Stewart, G. N. (1, 3, 4) May 28, 1931.
Stiles, Percy G. (1) July 5, 1936.
Straus, Henry W. (6) 1937.
Terry, Oliver P. (1) December 6, 1933.
Thatcher, Roscoe Wilfred (2) December 6, 1933.
Thompson, Wm. G. (1) October 27, 1927.
Trask, James D. (6) May 24, 1942.
Underhill, Frank P. (1, 2, 3) June 28, 1932.
Van Slyke, Lucius L. (2) September 30, 1931.
Vaughan, Victor C. (1, 4) October 21, 1929.
Vincent, S. (1) December 31, 1933.
Von Brücke, Ernest T. (1) June 12, 1941.
von Voit, C. (1h) January 31, 1908.
Walton, D. C. (3) March 6, 1942.
Warren, Joseph W. (1) December 20, 1916.
Warthin, Aldred Scott (4) May 23, 1931.
Webster, Ralph W. (2) July 2, 1930.
Weil, Richard (3, 6) November 19, 1917.
Weiss, Soma (3) January 31, 1942.
Welch, William H. (1, 4h) April 30, 1934.
Westbrook, Frank F. (1) October 21, 1918.
Wherry, William Buchanan (4) November 1, 1936.
Wiley, Harvey W. (2) June 30, 1930.
Woelfel, A. (1) January 31, 1920.
Wood, Horatio C. (1) January 3, 1920.
Zinsser, Hans (4, 6) September 4, 1940.

INDEX

ABSTRACTS, corrections, 201.
Almquist, H. J. The amino acid requirements and protein metabolism of the avian organism, 269.
American Association of Immunologists, 373.
American Institute of Nutrition, 370.
American Physiological Society, 357.
American Society for Experimental Pathology, 369.
American Society for Pharmacology and Experimental Therapeutics, 365.
American Society of Biological Chemists, 360.
Amino acids in hemoglobin formation, 219.
Anesthesia, clinical, newer viewpoints on, 213.

BERG, C. P. The metabolism of the *d*-amino acids, 281.
Bing, F. C. Foods as sources of the vitamins, 296.
——. Guiding principles for the fortification of foods, 336.
Boston meeting, notes on, 201.
Burr, G. O. Significance of the essential fatty acids, 224.

CLEVELAND Meeting, notice of, 353.
Cohen, P. P. Transamination, 273.

DAWSON, A. B. Some morphological aspects of the secretory process, 233.
Doisy, E. A., S. A. Thayer and J. T. van Bruggen. Metabolism of the estrogens, 202.

ELVEHJEM, C. A. The biological action of the vitamins, 304.
Estrogens, metabolism of, 202.
Executive Committee, 1942, 353.

FATTY acids, essential, 224.
Federation By-laws, 356.
—— Placement Service, 357.
Food consumption studies and dietary recommendations, 327.
——, definitions and standards of identity for, 324.
——, requirements, clinical observations bearing on, 330.
Foods, enriched, laboratory control of, 343.
——, guiding principles for the fortification of, 336.

GASTRIC glands, acid formation by, 255.
Goodhart, R. See Jolliffe and Goodhart, 316.
Gordon, E. S. Clinical observations bearing on food requirements, 330.
Gray, J. S. The formation of acid by the gastric glands, 255.

HEMOGLOBIN formation, amino acids in, 219.
Höber, R. Physicochemical aspects of the secretory process, 240.

JOLLIFFE, N. and R. Goodhart. Vitamins in the practice of medicine, 316.

KING, C. G. Some specific physiological disturbances induced by marginal vitamin deficiencies (C and B_1), 293.

MEMBERS, 375.
——, deceased, 438.
——, honorary, 374.
Membership, summary of, 438.
Metabolism of the aromatic amino acids, relation of vitamin C to, 287.
—— of the *d*-amino acids, 281.
Molitor, H. Vitamins as pharmacological agents, 309.
Murlin, J. R. Nutritional problems in relation to the nation's health, 209.

NELSON, E. M. Definitions and standards of identity for food, 324.
Nutritional problems and the nation's health, 209.

OSER, B. L. The laboratory control of enriched foods, 343.

PROTEIN metabolism of avian organism, amino acid requirements and, 269.

ROBSCHEIT-ROBBINS, F. S. Amino acids in hemoglobin formation, 219.

SEALOCK, R. R. The relation of vitamin C to the metabolism of the aromatic amino acids, 287.
Sebrell, W. H. Vitamins and public health, 319.
Secretion of dyestuffs by gastric glands and pancreas, 246.
——, zymogen, 261.
Secretory process, morphological aspects of, 233.
—— ——, physicochemical aspects of, 240.
Spies, T. D., Chairman, Symposium on deficiency diseases, 292.
Stiebeling, H. K. Food consumption studies and dietary recommendations, 327.

THAYER, S. A. See Doisy, Thayer and Van Bruggen, 202.
Thomas, J. E. The effect of nervous and hormonal stimulation on zymogen secretion, 261.
Transamination, 273.

VAN BRUGGEN, J. T. See DOISY, THAYER and VAN BRUGGEN, 202.
VISSCHER, M. B. The secretion of dyestuffs by the gastric glands and the pancreas, 246.
Vitamin deficiencies, marginal, some specific physiological disturbances induced by, 293.
Vitamins and public health, 319.
Vitamins as pharmacological agents, 309.
——, biological action of, 304.
——, foods as sources of, 296.
—— in the practice of medicine, 316.

WATERS, R. M. Newer viewpoints on clinical anesthesia, 213.

OHIO STATE UNIVERSITY
SEP 11 1944
LIBRARY

B & Z Library
QH 301
F29
v.1

VOLUME 1 NUMBER 1

FEDERATION PROCEEDINGS

PART I

Published quarterly by the

FEDERATION OF AMERICAN SOCIETIES

for EXPERIMENTAL BIOLOGY

The American Physiological Society
The American Society of Biological Chemists
The American Society for Pharmacology and Experimental Therapeutics
The American Society for Experimental Pathology
The American Institute of Nutrition

Contents

PROGRAM OF

TWENTY-NINTH ANNUAL MEETING

BOSTON, MASS.

MARCH 31, APRIL 1, 2, 3, 4, 1942

QH
301
F29
v.1

Issued March 16, 1942

Baltimore, U. S. A.

Price 50 cents

Motion Pictures, Salon, The Copley-Plaza, Thursday and Friday

Static Demonstrations, Mezzanine, Hotel Statler, Daily 9 a.m. to 5 p.m.,

PHYSIOLOGY: STATLER

Time	Ball Room	A Ball Room Assembly	B Georgian	C Parlor A	D Parlors B-C	E Salle Moderne
Wed., 9 a.m.						
Wed., 2 p.m.						
Wed., 8 p.m.						
Thurs., 10 a.m.	JOINT SESSION					
Thurs., 2 p.m.		Electrophysiol. EEG	Heart 4:30 Bus.	Blood	Cellular Metabolism	Endocrines
Thurs., Eve.	8:30 p.m.: SMOKER					
Fri., 9 a.m.		Neurophysiology	Cardiodynamics Bus. 11:30	Liver Water Balance	Metabolism	Adrenals
Fri., 2 p.m.		Brain	Shock and Allied Problems	Diabetes and Allied Problems	Respiration	Gastro-intestinal
Fri., 8 p.m.						
Sat., 9 a.m.	Symp.: Secretion (Section D)	Vision Hearing	Blood Flow Blood Pressure	Muscle Physiology		Sex Hormones

BIOCHEMISTRY, NUTRITION: COPLEY-PLAZA

Time	Ball Room	Foyer	Swiss	Sheraton	Salon
Wed., 9 a.m.	**Nutrition** Gen. Session 11:30 Bus.				
Wed., 2 p.m.	**Nutrition** Gen. Session 5:00 Bus.				
Wed., 8 p.m.		**Nutrition E** Symp.: Fortified Foods	**Nutrition B** Symp.: Vitamin Requirements		**Nutrition C** Symp.: Dietary and Blood Proteins
Thurs., 10 a.m.	JOINT SESSION AT STATLER				MOTION PICTURE DEMONSTRATIONS
Thurs., 2 p.m.		**Biochem. A** Symp.: Enzymes and Vitamins 5:00 Bus.	**Biochem. B** Amino Acids	**Biochem. C** Carbon Metab.	
Thurs. Eve.	SMOKER AT STATLER				
Fri., 9 a.m.	**Biochem. C** Steroids and Hormones	**Biochem. A** Symp.: N. Metabolism 11:30 Bus.	**Biochem. B** Fats & Phospholipids; Electrophoresis		
Fri., 2 p.m.		**Biochem. A** Symp.: Physics and Biochem.	**Biochem. B** Amino Acids	**Biochem. C** Tissue Oxidation	
Fri., 8 p.m.			**Biochem.** Joint Session with Am. Stat. Assoc.		
Sat., 9 a.m.		**Biochem. A** Electrolytes and Gen. papers	**Biochem. B** Proteins and Enzymes	**Biochem. C** Vitamins	

NUTRITION: LENOX

Dome	Monaco
Nutrition D Symp: Dehydr'n and Nutr. Value	**Nutrition A** Symp.: Bones and Teeth
JOINT SESSION AT STATLER	
Nutrition Scien. Session	
SMOKER AT STATLER	

Motion Pictures, Salon, The Copley-Plaza, Thursday and Friday

Static Demonstrations, Mezzanine, Hotel Statler, Daily 9 a.m. to 5 p.m.

PHARMACOLOGY ; BRUNSWICK

Time	A Ball Room	B Casino
Wed., 9 a.m.		
Wed., 2 p.m.	Analgesics; Opiates	Autonomic and Rel. Drugs
Wed., 8 p.m.	Symp.: Morphine Problems	
Thurs., 10 a.m.	JOINT SESSION AT STATLER	
Thurs., 2 p.m.	Toxicity; Tolerance	Gen. Anesthetics Hypnotics
	4:30 Bus.	
Thurs., Eve.	6:30 Dinner at University Club 8:30 SMOKER AT STATLER	
Fri., 9 a.m.	Cardiac; Glycosides	Chemotherapy; Amines; Circ. Drugs
	11:30 Bus.	
Fri., 2 p.m.	Symp.: Deficiency Dis.	
Sat., 9 a.m.	Hormones; Excretion; Convulsants	Sulfonamides

PATHOLOGY; PARKER HOUSE

	Hawthorne	B
Tues. and Wed.	Meetings of American Society of Immunologists and Amer. Association for Cancer Research	
	JOINT SESSION AT STATLER	
	Joint Session with Immunol.	
	5:00 Bus.	
	SMOKER AT STATLER	
	Vitamins Microbiology	
	Hematology A	Neoplasms Hyperplasia B
	Kidney and Circulation General	

MEMBERS DECEASED

BODANSKY, MEYER (*2*), June 14, 1941
CONNOR, C. L. (*4*), June 12, 1941
COULTER, C. B. (*4*), May 10, 1940
D'AUNOY, J. R. (*4*), Sept. 17, 1941
HENDERSON, L. J. (*1, 2*), Feb. 10, 1942
KRISS, MAX (*5*) Nov. 5, 1941
LEECH P. N. (*3*), Jan. 14, 1941
LEWIS, DEAN (*1*), Oct. 9, 1941
MALLORY, F. B. (*4*), Sept. 28, 1941
MILLER, E. S. (*2*), June 11, 1941
PERLA, DAVIS (*4*), June 14, 1940
SCHOENHEIMER, RUDOLF (*2*), Oct. 14, 1941
VON BRÜCKE, ERNEST TH. (*1*), June 12, 1941

FEDERATION EXECUTIVE COMMITTEE

1941-1942

Philip Bard, Carl J. Wiggers, *Physiology*

Rudolph J. Anderson, Arnold K. Balls, *Biochemistry*

E. M. K. Geiling, Raymond N. Bieter, *Pharmacology*

Jesse L. Bollman, H. P. Smith, *Pathology*

Albert G. Hogan, Arthur H. Smith, *Nutrition*

Albert G. Hogan, *Chairman*

Shields Warren, *Ex-Chairman*

D. R. Hooker, *Secretary*

BOSTON LOCAL COMMITTEE

1941-1942

Shields Warren, *Chairman*

Hallowell Davis, *Secretary*

David Rapport, *Treasurer*

H. K. Beecher

D. L. Belding

T. M. Carpenter

G. P. Grabfield

H. Hoagland

R. G. Hoskins

M. O. Lee

T. Mallory

F. H. Pratt

QH 301
F29
v.1
B & Z Library
B+Z.

VOLUME 1 NUMBER 1

OHIO STATE
UNIVERSITY
OCT 1 1942
LIBRARY

FEDERATION PROCEEDINGS

PART II

Published quarterly by the

FEDERATION OF AMERICAN SOCIETIES

for EXPERIMENTAL BIOLOGY

The American Physiological Society
The American Society of Biological Chemists
The American Society for Pharmacology and Experimental Therapeutics
The American Society for Experimental Pathology
The American Institute of Nutrition

Contents

TWENTY-NINTH ANNUAL MEETING

ABSTRACTS OF PAPERS IN:

Physiology 1
Biochemistry 97
Pharmacology 141
Pathology 173
Nutrition 187

INDEX OF AUTHORS 193
INDEX OF SUBJECTS 196

Issued March 16, 1942

Baltimore, U. S. A.

Price $1.50

FEDERATION PROCEEDINGS

Published by the

FEDERATION OF AMERICAN SOCIETIES

FOR EXPERIMENTAL BIOLOGY

COMPOSED OF

The American Physiological Society

The American Society of Biological Chemists

The American Society for Pharmacology and Experimental Therapeutics

The American Society for Experimental Pathology

The American Institute of Nutrition

Editorial Board

W. O. FENN, *Chairman* — MORTON MCCUTCHEON

PHILIP BARD — C. F. SCHMIDT

C. G. KING — A. H. SMITH

D. R. HOOKER, *Managing Editor*

19 West Chase St., Baltimore, Md.

THE FEDERATION PROCEEDINGS is published quarterly by the Federation of American Societies for Experimental Biology, 19 West Chase St., Baltimore, Md., to make a volume of about six hundred pages. The *March* issue will contain the abstracts of papers to be presented at the scientific sessions of the Federation. These abstracts will be segregated as to Societies and indexed as to authors and subjects. The *June, September and December* issues will contain matter pertinent to the Federation membership and symposium and other special papers presented at Federation meetings as selected by the Editorial Board. The volume will be adequately indexed.

The subscription price will be $4.00 ($4.75 foreign) payable in advance. Single issues may be purchased, if ordered in advance, at prices to be determined at the time of issue. Subscriptions and orders should be sent to the Managing Editor.

Application for entry as second-class matter pending at the Post Office at Baltimore, Maryland, under the act of March 3, 1879

OHIO STATE UNIVERSITY
SEP 11 1944
LIBRARY

B & Z Library
QH 301
F29
v.1

VOLUME 1 NUMBER 2

FEDERATION PROCEEDINGS

Published quarterly by the

FEDERATION OF AMERICAN SOCIETIES

for EXPERIMENTAL BIOLOGY

The American Physiological Society
The American Society of Biological Chemists
The American Society for Pharmacology and Experimental Therapeutics
The American Society for Experimental Pathology
The American Institute of Nutrition
The American Association of Immunologists

Contents

Abstracts, Corrections of Errors in 201
Notes on Boston Meeting 201
Metabolism of Estrogens 202
Nutritional Problems in Relation to the Nation's Health 209
Newer Viewpoints on Clinical Anesthesia 213
Amino Acids in Hemoglobin Formation 219
Significance of the Essential Fatty Acids 224
Some Morphological Aspects of the Secretory Process 233
Physicochemical Aspects of the Secretory Process 240
The Secretion of Dyestuffs by the Gastric Glands and Pancreas . 246
The Formation of Acid by the Gastric Glands 255
The Effect of Nervous and Hormonal Stimulation on Zymogen Secretion 261

Issued June, 1942

Baltimore, U. S. A.

FEDERATION PROCEEDINGS

Published by the

FEDERATION OF AMERICAN SOCIETIES
FOR EXPERIMENTAL BIOLOGY

COMPOSED OF

The American Physiological Society

The American Society of Biological Chemists

The American Society for Pharmacology and Experimental Therapeutics

The American Society for Experimental Pathology

The American Institute of Nutrition

The American Association of Immunologists

Editorial Board

PHILIP BARD, *Chairman*

C. G. KING — C. F. SCHMIDT

MORTON MCCUTCHEON — A. H. SMITH

D. R. HOOKER, *Managing Editor*

19 West Chase St., Baltimore, Md.

THE FEDERATION PROCEEDINGS is published quarterly by the Federation of American Societies for Experimental Biology, 19 West Chase St., Baltimore, Md., to make a volume of about six hundred pages. The *March* issue will contain the abstracts of papers to be presented at the scientific sessions of the Federation. These abstracts will be segregated as to Societies and indexed as to authors and subjects. The *June, September and December* issues will contain matter pertinent to the Federation membership and symposium and other special papers presented at Federation meetings as selected by the Editorial Board. The volume will be adequately indexed.

The subscription price will be $4.00 ($4.75 foreign) payable in advance. Single issues may be purchased, if ordered in advance, at prices to be determined at the time of issue. Subscriptions and orders should be sent to the Managing Editor.

Application for entry as second-class matter pending at the Post Office at Baltimore, Maryland, under the act of March 3, 1879

OHIO STATE UNIVERSITY
SEP 11 1944
LIBRARY

B & Z Library
QH 301
F29
v.1

VOLUME 1 NUMBER 3

FEDERATION PROCEEDINGS

Published quarterly by the

FEDERATION OF AMERICAN SOCIETIES

for EXPERIMENTAL BIOLOGY

The American Physiological Society
The American Society of Biological Chemists
The American Society for Pharmacology and Experimental Therapeutics
The American Society for Experimental Pathology
The American Institute of Nutrition
The American Association of Immunologists

Contents

The Amino Acid Requirements and Protein Metabolism of the Avian Organism 269
Transamination 273
The Metabolism of the *d*-Amino Acids 281
The Relation of Vitamin C to the Metabolism of the Aromatic Amino Acids 287
Some Specific Physiological Disturbances Induced by Marginal Vitamin Deficiencies (C and B_1) 293
Foods as Sources of the Vitamins 296
The Biological Action of the Vitamins 304
Vitamins as Pharmacological Agents 309
Vitamins in the Practice of Medicine 316
Vitamins and Public Health 319
Definitions and Standards of Identity for Food 324
Food Consumption Studies and Dietary Recommendations 327
Clinical Observations Bearing on Food Requirements 330
Guiding Principles for the Fortification of Foods 336
The Laboratory Control of Enriched Foods 343

Issued September, 1942

Baltimore, U. S. A.

FEDERATION PROCEEDINGS

Published by the

FEDERATION OF AMERICAN SOCIETIES
FOR EXPERIMENTAL BIOLOGY

COMPOSED OF

The American Physiological Society

The American Society of Biological Chemists

The American Society for Pharmacology and Experimental Therapeutics

The American Society for Experimental Pathology

The American Institute of Nutrition

The American Association of Immunologists

Editorial Board

PHILIP BARD, *Chairman*

C. G. KING — C. F. SCHMIDT

MORTON MCCUTCHEON — A. H. SMITH

D. R. HOOKER, *Managing Editor*

19 West Chase St., Baltimore, Md.

THE FEDERATION PROCEEDINGS is published quarterly by the Federation of American Societies for Experimental Biology, 19 West Chase St., Baltimore, Md. The *March* issue will contain the abstracts of papers to be presented at the scientific sessions of the Federation. These abstracts will be segregated as to Societies and indexed as to authors and subjects. The *June, September and December* issues will contain matter pertinent to the Federation membership and symposium and other special papers presented at Federation meetings as selected by the Editorial Board. The volume will be adequately indexed.

The subscription price will be $4.00 ($4.75 foreign) payable in advance. Single issues may be purchased, if ordered in advance, at prices to be determined at the time of issue. Subscriptions and orders should be sent to the Managing Editor.

Entered as second-class matter March 16, 1942, at the Post Office at Baltimore, Maryland, under the Act of August 24, 1912.

OHIO STATE UNIVERSITY
SEP 11 1944
LIBRARY

B & Z Library
QH 301
F29
v.1

VOLUME 1 NUMBER 4

FEDERATION PROCEEDINGS

Published quarterly by the

FEDERATION OF AMERICAN SOCIETIES

for EXPERIMENTAL BIOLOGY

The American Physiological Society
The American Society of Biological Chemists
The American Society for Pharmacology and Experimental Therapeutics
The American Society for Experimental Pathology
The American Institute of Nutrition
The American Association of Immunologists

Contents

Announcements of the Cleveland Meeting 353
Federation of American Societies for Experimental Biology 353
Executive Committee, 1942 353
Former Executive Committees 353
By-Laws of the Federation 356
Placement Service 357
The American Physiological Society, 1942 357
The American Society of Biological Chemists, 1942 360
The American Society for Pharmacology and Experimental Therapeutics, 1942 365
The American Society for Experimental Pathology, 1942 368
The American Institute of Nutrition, 1942 370
The American Association of Immunologists, 1942 373
Membership List of All Societies, 1942 374
Summary of Membership, 1942 438
Members Deceased, 1942 438
Index 441

Issued December, 1942

Baltimore, U. S. A.

FEDERATION PROCEEDINGS

Published by the

FEDERATION OF AMERICAN SOCIETIES
FOR EXPERIMENTAL BIOLOGY

COMPOSED OF

The American Physiological Society

The American Society of Biological Chemists

The American Society for Pharmacology and Experimental Therapeutics

The American Society for Experimental Pathology

The American Institute of Nutrition

The American Association of Immunologists

Editorial Board

PHILIP BARD, *Chairman*

C. G. KING — MORTON MCCUTCHEON

ARTHUR LOCKE — C. F. SCHMIDT

A. H. SMITH

D. R. HOOKER, *Managing Editor*

19 West Chase St., Baltimore, Md.

THE FEDERATION PROCEEDINGS is published quarterly by the Federation of American Societies for Experimental Biology, 19 West Chase St., Baltimore, Md. The *March* issue will contain the abstracts of papers to be presented at the scientific sessions of the Federation. These abstracts will be segregated as to Societies and indexed as to authors and subjects. The *June, September and December* issues will contain matter pertinent to the Federation membership and symposium and other special papers presented at Federation meetings as selected by the Editorial Board. The volume will be adequately indexed.

The subscription price will be $4.00 ($4.75 foreign) payable in advance. Single issues may be purchased, if ordered in advance, at prices to be determined at the time of issue. Subscriptions and orders should be sent to the Managing Editor.

Entered as second-class matter March 16, 1942, at the Post Office at Baltimore, Maryland, under the Act of August 24, 1912.